Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB,JPAB,EPAB; PLUR=YES; OP=ADJ			
L12	(cd40L or cd40 adj ligand or gp39) same (antibod$) and('lfa-1' or cd11a$) same (antibod$) and (transplant$ or graft$)	45	L12
DB=USPT,DWPI; PLUR=YES; OP=ADJ			
L11	(cd40L or cd40 adj ligand or gp39) same (antibod$) and('lfa-1' or cd11a$) same (antibod$) and (transplant$ or graft$)	18	L11
L10	(ctla$) same (antibod$) and('lfa-1' or cd11a$) same (antibod$) and (transplant$ or graft$)	43	L10
L9	(ctla$) same (antibod$) and(lfa$ or cd11a$) same (antibod$) and (transplant$ or graft$)	59	L9
L8	(ctla$) same (antibod$) same(lfa$ or cd11a$) same (antibod$) and (transplant$ or graft$)	30	L8
L7	(ctla$) same (antibod$) same(cd40L or cd40 adj ligand or gp39) same (antibod$) and (transplant$ or graft$)	57	L7
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L6	(ctla$) same (antibod$) same(cd40L or cd40 adj ligand or gp39) same (antibod$) and (transplant$ or graft$)	147	L6
L5	(lfa or cdl1a)same (antibod$) and (ctla$) same (antibod$) same(cd40L or cd40 adj ligand or gp39) same (antibod$) and (transplant$ or graft$)	17	L5
L4	L3 and (graft$ or transplant$)	11	L4
L3	(lfa or cdl1a) same (ctla$) same (cd40L or cd40 adj ligand or gp39) same (antibod$)	14	L3
L2	L1 and (ctla$ or lfa$ or cd40L or cd40 adj ligand or cd154)	5	L2
L1	townsend.in.	2150	L1

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Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,DWPI; PLUR=YES; OP=ADJ			
L9	(ctla$) same (antibod$) and(lfa$ or cd11a$) same (antibod$) and (transplant$ or graft$)	59	L9
L8	(ctla$) same (antibod$) same(lfa$ or cd11a$) same (antibod$) and (transplant$ or graft$)	30	L8
L7	(ctla$) same (antibod$) same(cd40L or cd40 adj ligand or gp39) same (antibod$) and (transplant$ or graft$)	57	L7
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L6	(ctla$) same (antibod$) same(cd40L or cd40 adj ligand or gp39) same (antibod$) and (transplant$ or graft$)	147	L6
L5	(lfa or cdl1a)same (antibod$) and (ctla$) same (antibod$) same(cd40L or cd40 adj ligand or gp39) same (antibod$) and (transplant$ or graft$)	17	L5
L4	L3 and (graft$ or transplant$)	11	L4
L3	(lfa or cdl1a) same (ctla$) same (cd40L or cd40 adj ligand or gp39) same (antibod$)	14	L3
L2	L1 and (ctla$ or lfa$ or cd40L or cd40 adj ligand or cd154)	5	L2
L1	townsend.in.	2150	L1

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L4: Entry 11 of 11 File: USPT Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5876950 A
TITLE: Monoclonal antibodies specific for different epitopes of human GP39 and methods for their use in diagnosis and therapy

Abstract Text (1):
The present invention provides monoclonal antibodies, antigen binding fragment and recombinant binding proteins specific for human gp39. These antibodies are specific for at least eight different epitopes on gp39. Hybridomas secreting specific antibodies which bind to these epitopes are also provided. Further, the present invention discloses the amino acid sequence of immunoglobulin light and heavy chain variable regions which bind to epitopes of gp39 and provide sFv and humanized antibodies which bind gp39. Also, provided are pharmaceutical compositions comprising the monoclonal antibodies, antigen binding fragments and recombinant binding proteins which bind gp39 and methods for using these compositions in diagnosing disease states, inhibiting B cell activation and for treating immunological disorders, such as autoimmune diseases, allergic responses, organ rejection and graft-versus-host disease. Antibodies of the present invention can also be used to image cells which express gp39 on their surface, such as tumor cells (e.g., lymphoma) and to target therapeutic agents to target cells.

Brief Summary Text (3):
A number of important T cell surface proteins involved in cell-cell interactions have been identified including CD2, CD4, CD8, CD28, LFA-1, CTLA-4 and gp39. These proteins participate in cell-cell contact by binding to their counter-receptors on APC and provide important costimulatory signals to T cells which modulate signals received through the T-cell antigen receptor. These costimulatory signals are necessary for the T cell to become fully engaged and express both membrane-bound and soluble factors required for the proper activation of the T cell-dependent effector cells (B cells, natural killer cells, monocytes, neutrophils, etc.). The gp39/CD40 T cell ligand/B cell receptor pair plays a critical role in the humoral immune response. In vitro studies have shown that this receptor/ligand pair is involved in B cell proliferation, antibody and cytokine production and cell viability. Studies in vivo, both through blocking with a monoclonal antibody or by observation of a genetic defect in gp39, have validated the in vitro results, and extended them to the requirement for a functional gp39 for germinal center formation during immune response to antigen.

Brief Summary Text (28):
Methods are also provided for using these pharmaceutical compositions to inhibit the activation of B cells in an animal by administering an effective amount of one of the compositions described above. The animal provided with the composition can include mice, rats, rabbits and humans. The inhibition of the activation of B cells can prevent an autoimmune response, the rejection of a transplanted organ, graft-versus-host disease, an allergic response or an inflammatory response. Autoimmune diseases preventable using this method can include psoriasis, rheumatoid arthritis, systemic lupus erythematosus or diabetes mellitus, among others.

Detailed Description Text (30):
The pharmaceutical compositions of the present invention find use in vivo to inhibit the CD40/gp39 interaction. Blocking this interaction limits both primary and secondary antibody responses to T-cell dependent antigens and antibody production specific for these antigens. Therefore, the monoclonal antibodies, antigen binding fragments, and

recombinant binding proteins can be used to inhibit the activation of B cells, modulating or inhibiting autoimmune disease (i.e., psoriasis, rheumatoid arthritis, systemic lupus erythematosis, diabetes mellitus, etc.), allergic responses, organ rejection or graft-versus-host disease. The compositions can also be used for imaging tumors which express gp39, when labeled with a detectable marker. When conjugated with a therapeutic agent or as a fusion protein with a therapeutic agent, the monoclonal antibodies, antigen binding fragment or recombinant binding proteins, can also be used o target the therapeutic agent to tumor cells.

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L5: Entry 16 of 17 File: USPT Apr 23, 2002

DOCUMENT-IDENTIFIER: US 6376459 B1
TITLE: Inhibiting B cell activation with soluble CD40 or fusion proteins thereof

Abstract Text (1):
The present invention relates to a counter-receptor, termed CD40CR, for the CD40 B-cell antigen, and to soluble ligands for this receptor, including fusion molecules comprising at least a portion of CD40 protein. It is based, at least in part, on the discovery that a soluble CD40/immunoglobulin fusion protein or antibody specific for gp39 on T cells was able to inhibit helper T-cell mediated B-cell activation by binding to a novel 39 kD protein receptor on helper T-cell membranes. The present invention provides for a substantially purified CD40CR receptor; for soluble ligands of CD40CR, including antibodies as well as fusion molecules comprising at least a portion of CD40 protein; and for methods of controlling B-cell activation which may be especially useful in the treatment of allergy or autoimmune disease, including graft-versus-host disease and rheumatoid arthritis.

Brief Summary Text (2):
The present invention relates to a counter-receptor, termed CD40CR, (also known as CD40 ligand) for the CD40 B-cell antigen, and to soluble ligands for this receptor, including fusion molecules comprising at least a portion of CD40 protein. It is based, at least in part, on the discovery that a soluble CD40/immunoglobulin fusion protein or antibody specific for gp39 on T cells was able to inhibit helper T-cell mediated B-cell activation by binding to a novel 39 kD protein receptor on helper T-cell membranes. The present invention provides for a substantially purified CD40CR receptor; for soluble ligands of CD40CR, including antibodies as well as fusion molecules comprising at least a portion of CD40 protein; and for methods of controlling B-cell activation which may be especially useful in the treatment of allergy or autoimmune disease, including graft-versus-host disease (GVHD) and rheumatoid arthritis.

Detailed Description Text (40):
For example, plasma membrane (PM) from activated (PM.sup.Act) but not resting (PM.sup.rest) T.sub.h cells was found to induce B-cell RNA synthesis (FIG. 1a); this induction, indicative of B-cell activation, was not affected by antibodies such as anti-LFA-1, anti-CD4, anti-ICAM-1. CD40-Ig or MR1, however, were found to be able to inhibit PM.sup.Act -induced B-cell activation, as shown in FIG. 1B and FIG. 6.

Detailed Description Text (58):
In additional embodiments of the invention, CD40CR ligand may be used to prevent or ameliorate graft versus host disease in a subject in need of such treatment. Accordingly, the invention provides for methods of inhibiting graft versus host disease in a subject in need of such treatment comprising an effective amount of CD40CR ligand to a subject in need of such treatment. See Sections 11 and 13 for data demonstrating that anti-gp39 antibody was able to inhibit grafted T cells from inducing host B cell activation.

Detailed Description Text (70):
In a further embodiment, CD40CR may be conjugated to a toxin, and then administered to a subject under circumstances in which it would be preferable to destroy B-cells that express CD40. Examples of such circumstances include patients receiving organ transplants or suffering from multiple myeloma or another B-cell malignancy,or from autoimmune disease.

Detailed Description Text (172):
For eliciting primary antibody responses to SRBC or TNP-SRBC, mice were immunized with 200 .mu.l of 1% SRBC or TNP-SRBC suspension (i.v.). The IgM, anti-SRBC response was assayed 5 d after administration of antigen using a modification of the Jerne plaque assay (Jerne et al. (1974) Transplant Rev. 18:130). IgM anti-TNP responses were measured by ELISA on day 6. Primary responses to the heterologous immunogobulin Chi-L6 were generated by i.p. immunization of 100 .mu.g Chi-L6, in alum, per mouse. The serum IgM anti-Chi-L6 antibody response was measured after 7 d. Primary responses to TNP-Ficoll were generated by immunization with 25 .mu.g of TNP-Ficoll i.p. The IgM anti-TNP response was measured on day 6 by ELISA.

Detailed Description Text (207):
The focus of the present study was to demonstrate the potential use of anti-gp39 in the control of TD humoral immunity. Brief treatment regimes with anti-gp39 resulted in prolonged suppression, an attractive attribute of this therapeutic antibody. Of special interest may be the capacity of anti-gp39 to prevent primary and secondary humoral responses to other heterologous, therapeutic antibodies such as Chi-L6. This would permit the exposure of patients to repeated adminstrations of heterologous therapeutic antibodies. Inhibitory effects on humoral immunity have been observed with other mAbs, i.e. anti-CD4 E (Carteron et al. (1990) Clin. Immunol. Pathol. 56:373; Horneff et al. (1991) Arthritis and rheumatism 34:129). Although it is unclear how anti-CD4 mediates immune suppression, extensive deletion of CD4+ T cells is correlated with suppressive efficacy (Shizuru et al. (1992) Immunol. Rev. 129:103), a phenomenon not observed with anti-gp39 therapy. In addition to anti-CD4, it has been shown that the interference by CTLA-4 of CD28 triggering, a co-stimulatory molecule on T.sub.h cells, also suppresses TD antibody responses (Linsley et al. (1992) Science 257:792) and blocks xenogeneic graft rejection (Lenschow et al. (1992) Science 257:789). Similar to anti-gp39 administration, CTLA-4 induced a state of prolonged immune suppression. Because anti-gp39 and CTLA-4 mediate their immunosuppressive effects at distinct stages of the humoral immune response, co-administration of these two immunosuppressive drugs may provide additive or synergistic immunosuppressive effects on immunity.

Detailed Description Text (287):
Reversal of the splenomegaly inhibition of hyper Ig production in GVH-induced mice by anti-gp 39 administration, indicates that anti-gp39 blocked the ability of the grafted T cells to induce host B cell activation (FIG. 33). This inhibition persists for 7 days even when the treatment is terminated. These results indicate that T cell function has been affected either by clonal deletion of the reactive T cells or that T cell anergy has occurred. Alternatively, inhibitory levels of anti-gp39 remain during this 7 day interval resulting in the blockade of Th effector function. This data suggests that anti-gp39 interferes with the ability of T cells to elicit a strong GVHD clinical immunopathology and splenomegaly.

Detailed Description Text (297):
Role of CD40-GP39 Interactions in Chronic Graft-Versus-Host Disease

Detailed Description Text (313):
In the mouse, one of the classical consequences of GVH reaction is the enlargement of the spleen. It is primarily the host's own cells that infiltrate and enlarge the spleen, although this is in response to the presence of the graft cells. It was observed, upon removal of the spleens from GVHD-induced mice, that GVHD resulted in splenomegaly. FIGS. 38A-38B indicates that mice induced with GVHD for 1 week and 2 weeks resulted in spleens with almost twice the cell numbers compared to normal F.sub.1 recipients. When mice were treated with the anti-gp39 antibody (250 .mu.g/mouse, days 0,2,4, and 6), the cell numbers returned to levels of the normal spleens and remained low for 1 week after the antibody treatment was terminated (2 wk GVHD+1 wk MR1 (FIG. 37)).

Detailed Description Text (318):
Reversal of splenomegaly and inhibition of hyper Ig production in GVH-induced mice by anti-gp39 administration, indicates that anti-gp39 blocked the ability of the grafted T cells to induce host B cell activation. This inhibition persists for 7 days even when the treatment is terminated. These results indicate that T cell function has been affected either by clonal deletion of the reactive T cells or that T cell anergy has

occurred. Alternatively, inhibitory levels of anti-gp39 remain during this 7 day interval resulting in the blockade of Th effector function. This data indicates that anti-gp39 interferes with the ability of T cells to elicit a strong GVHD clinical immunopathology and splenomegaly.

Other Reference Publication (3):
Larsen et al. Transplantation 61: 4-9 (1996).*

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L7: Entry 10 of 57 File: USPT Feb 4, 2003

DOCUMENT-IDENTIFIER: US 6514513 B1
TITLE: Costimulatory blockade and mixed chimerism in transplantation

Abstract Text (1):
Use of blockade of costimulation and hematopoietic stem cell transplantation in the promotion of graft acceptance.

Brief Summary Text (2):
The invention relates to tissue and organ transplantation.

Brief Summary Text (3):
The field of organ transplantation has enjoyed substantial progress during the last two decades, resulting in marked improvements in short-term graft survival. Organ transplant recipients, however, still face substantial risks of long-term morbidity and mortality. Though modern immunosuppressive regimens have led to a dramatic reduction of the incidence of acute rejection episodes, they have yet to achieve a similar effect for chronic rejection, which is still the leading cause of graft loss during long-term follow-up. In addition, the requirement for life-long immunosuppressive drug therapy carries a significant risk of severe side effects, including tumors, infections and metabolic disorders. The reliable induction of donor-specific tolerance would solve both problems by obviating the need for chronic non-specific immunosuppression and by abrogating detrimental immunological reactions against the allograft.

Brief Summary Text (6):
Accordingly, the invention features a method of promoting acceptance, by a recipient mammal, of a graft from a donor mammal of a second species. The method includes: administering to the recipient, an inhibitor, e.g., a blocker, of a costimulatory pathway, (e.g., one or both of, an inhibitor, e.g., a blocker, of the CD40 ligand-CD40 interaction and an inhibitor, e.g., a blocker, of the CD28-B7 interaction); introducing, e.g., by intravenous injection, into the recipient mammal, hematopoietic stem cells, e.g., a bone marrow preparation; and preferably, implanting the graft in the recipient. The hematopoietic cells are believed to prepare the recipient for the graft that follows, by inducing tolerance at both the B-cell and T-cell levels.

Brief Summary Text (9):
In preferred embodiments CTLA4-Ig and an anti-CD40L antibody are administered.

Brief Summary Text (10):
In preferred embodiments, a blocker of the CD40/CD40L interaction, e.g., an anti-CD40L antibody is administered prior to administration of a blocker of the CD28/B7 interaction, e.g., CTLA4/Ig. The CD40/CD40L blocker can be administered on the day donor tissue is introduced and the CD28/B7 blocker administered 2, 3,4 5 or more days later.

Brief Summary Text (11):
The recipient mammal can be, by way of example, a human. The donor mammal can be, by way of example, a swine, e.g., a miniature swine. The graft is preferably from a discordant species. The graft preferably expresses a major histocompatibility complex (MHC) antigen, preferably a class II antigen. In particularly preferred embodiments the recipient is a primate, e.g., a human, and the donor is a swine, e.g., a miniature swine.

Brief Summary Text (19):
Repeated stem cell administration can promote engraftment, mixed chimerism, and preferably long-term deletional tolerance in graft recipients. Thus, the invention

also includes methods in which multiple hematopoietic stem cell administrations are provided to a recipient. Multiple administration can substantially reduce or eliminate the need for hematopoietic space-creating irradiation. Administration can be given prior to, at the time of, or after graft implantation. In preferred embodiments multiple administrations of stem cells are provided prior to the implantation of a graft. Two, three, four, five, or more administrations can be provided. The period between administrations of hematopoietic stem cells can be varied. In preferred embodiments a subsequent administration of hematopoietic stem cell is provided: at least two days, one week, one month, or six months after the previous administration of stem cells; when the recipient begins to show signs of host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain a level of mixed chimerism sufficient to maintain tolerance to donor antigen.

Brief Summary Text (20):
One or more post graft-implantation-administrations of donor stem cells can also be provided to minimize or eliminate the need for irradiation. Post graft administration of hematopoietic stem cells can be provided: at least two days, one week, one month, or six months after the previous administration of stem cells; at least two days, one week, one month, six months, or at any time in the life span of the recipient after the implantation of the graft; when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the host donor specific antibody response, or by a change in the host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a graft.

Brief Summary Text (22):
Although methods in which blockers of both pathways are administered may usually minimize or eliminate the need for other preparative steps, some embodiments include inactivating natural killer cells, preferably graft reactive or xenoreactive, e.g., swine reactive, NK cells, of the recipient mammal. This can be accomplished, e.g., by introducing into the recipient mammal an antibody capable of binding to natural killer cells of the recipient mammal, e.g., an anti-CD2 antibody, e.g., MEDI-507. The administration of antibodies, or other treatment to inactivate natural killer cells, can be given prior to introducing the hematopoietic stem cells into the recipient mammal or prior to implanting the graft in the recipient. This antibody can be the same or different from an antibody used to inactivate T cells.

Brief Summary Text (23):
Although methods in which blockers of both pathways are administered may usually minimize or eliminate the need for other preparative steps, some embodiments include inactivating T cells, preferably graft reactive or xenoreactive, e.g., swine reactive, T cells of the recipient mammal. This can be accomplished, e.g., by introducing into the recipient mammal an antibody capable of binding to T cells of the recipient mammal. The administration of antibodies, or other treatment to inactivate T cells, can be given prior to introducing the hematopoietic stem cells into the recipient mammal or prior to implanting the graft in the recipient. This antibody can be the same or different from an antibody used to inactivate natural killer cells.

Brief Summary Text (24):
One source of anti-NK antibody is anti-human thymocyte polyclonal anti-serum. Preferably, a second anti-mature T cell antibody can be administered as well, which lyses T cells as well as NK cells. Lysing T cells is advantageous for both bone marrow and graft survival. Anti-T cell antibodies are present, along with anti-NK antibodies, in anti-thymocyte anti-serum. Repeated doses of antibodies, e.g., anti-NK or anti-T cell antibodies, may be preferable. Monoclonal preparations can be used in the methods

of the invention. An anti-CD2 antibody, e.g., MEDI-507, can be used as an anti-NK antibody.

Brief Summary Text (26):
Although methods in which blockers of both pathways are administered may usually minimize or eliminate the need for other preparative steps, some embodiments include the inactivation of thymocytes or T cells which can be performed prior to hematopoietic stem cell or graft transplantation. In preferred embodiments the method includes diminishing or inhibiting thymocyte or T cell activity, preferably the activity of thymic or lymph node T cells by administering to the recipient a short course of an immunosuppressive agent, e.g., a chemical or drug, e.g., cyclosporine, sufficient to inactivate thymocytes or T cells, preferably thymic or lymph node T cells. The duration of the short course of immunosuppressive agent is: approximately equal to or less than 30, 40, 50, 60, 120, or 365 days; approximately equal to or less than 8-12 days, preferably about 10 days; approximately equal to or less than two, three, four, five, or ten times the 8-12 or 10 day period. The short course can begin: before or at about the time the treatment to induce tolerance is begun, e.g., at about the time stem cells are introduced into the recipient; on the day the treatment to induce tolerance is begun, e.g., on the day stem cells are introduced into the recipient; within 1, 2, 4, 6, 8, 10, or 30 days before or after the treatment to induce tolerance is begun, e.g., within 1, 2, 4, 6, 8, 10, or 30 days before or after stem cells are introduced into the recipient. The short course of an immunosuppressive can be administered in conjunction with an anti-T cell antibody The short course of an immunosuppressive should be sufficient in concentration and duration to inactivate T cells, e.g., thymic or lymph node T cells, which would not be inactivated by antibody-based inactivation of T cells, e.g., inactivation by intravenous administrations of ATG antibody, or similar, preparations.

Brief Summary Text (27):
Although methods in which blockers of both pathways are administered may usually minimize or eliminate the need for other preparative steps, some embodiments include (optionally): the step of, prior to hematopoietic stem cell transplantation, creating hematopoietic space, e.g., by irradiating the recipient mammal with low dose, e.g., less than 400, preferably less than 300, more preferably less than 200 or 100 cGy, whole body irradiation to deplete or partially deplete the bone marrow of the recipient. As is discussed herein this treatment can be reduced or entirely eliminated. Other methods of creating hematopoietic space, e.g., administering hematopoietic space creating antibodies or drugs, e.g., cyclophosphamide or busulfan, to the recipient, can be used. E.g., hematopoietic space can be formed by administering an inhibitor of cell proliferation, e.g., DSG, or an anti-metabolite, e.g. brequinar, or an anti-T cell antibody, e.g., one or both of an anti-CD4 or anti-CD8 antibody.

Brief Summary Text (28):
Other preferred embodiments include: the step of, preferably prior to hematopoietic stem cell transplantation, depleting natural antibodies from the blood of the recipient mammal. Depletion can be achieved, by way of example, by contacting the recipients blood with an epitope which absorbs preformed anti-donor antibody. The epitope can be coupled to an insoluble substrate and provided, e.g., as an affinity column. E.g., an .alpha.1-3 galactose linkage epitope-affinity matrix, e.g., matrix bound linear B type VI carbohydrate, can be used to deplete natural antibodies. Depletion can also be achieved by hemoperfusing an organ, e.g., a liver or a kidney, obtained from a mammal of the donor species. (In organ hemoperfusion antibodies in the blood bind to antigens on the cell surfaces of the organ and are thus removed from the blood.)

Brief Summary Text (29):
Other preferred embodiments include those in which: the same mammal of the second species is the donor of one or both the graft and the hematopoietic cells; and the antibody is an anti-human thymocyte polyclonal anti-serum, obtained, e.g., from a horse or pig.

Brief Summary Text (30):
In preferred embodiments, the method includes the step of introducing into the recipient a graft obtained from the donor which is obtained from a different organ

than the hematopoietic stem cells, e.g., a heart, pancreas, liver, or kidney.

Brief Summary Text (31):
In a particularly preferred embodiment the method includes: administering to a human recipient one or both, of an inhibitor, e.g., a blocker, of the CD40 ligand-CD40 interaction and an inhibitor, e.g., a blocker, of the CD28-B7 interaction; introducing, e.g., by intravenous injection, into the recipient mammal, hematopoietic stem cells, e.g., a bone marrow preparation; and implanting the graft in the recipient. The method can be practiced without T cell depletion or inactivation, with T cell depletion or inactivation, or with partial T cell depletion or inactivation. T cell inactivation can be effected by the administration of thymic irradiation, or anti T cell antibodies.

Brief Summary Text (33):
In another aspect, the invention features a method of promoting acceptance, by a recipient mammal, of a graft from a donor mammal of the same species. The method includes: administering to the recipient, an inhibitor, e.g., a blocker, of a costimulatory pathway, e.g., a blocker of the CD28-B7 interaction; introducing, e.g., by intravenous injection, into the recipient mammal, hematopoietic stem cells, e.g., a bone marrow preparation; and preferably, implanting the graft in the recipient. The hematopoietic cells are believed to prepare the recipient for the graft that follows, by inducing tolerance at both the B-cell and T-cell levels.

Brief Summary Text (43):
Repeated stem cell administration can promote engraftment, mixed chimerism, and preferably long-term deletional tolerance in graft recipients. Thus, the invention also includes methods in which multiple hematopoietic stem cell administrations are provided to a recipient. Multiple administration can substantially reduce or eliminate the need for hematopoietic space-creating irradiation. Administrations can be given prior to, at the time of, or after graft implantation. In preferred embodiments multiple administrations of stem cells are provided prior to the implantation of a graft. Two, three, four, five, or more administrations can be provided. The period between administrations of hematopoietic stem cells can be varied. In preferred embodiments a subsequent administration of hematopoietic stem cell is provided: at least two days, one week, one month, or six months after the previous administration of stem cells; when the recipient begins to show signs of host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain a level of mixed chimerism sufficient to maintain tolerance to donor antigen.

Brief Summary Text (44):
One or more post graft-implantation-administrations of donor stem cells can also be provided to minimize or eliminate the need for irradiation. Post graft administration of hematopoietic stem cells can be provided: at least two days, one week, one month, or six months after the previous administration of stem cells; at least two days, one week, one month, six months, or at any time in the life span of the recipient after the implantation of the graft; when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the host donor specific antibody response, or by a change in the host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a graft.

Brief Summary Text (46):
Although methods in which blockers of both pathways are administered may usually eliminate the need for other preparative steps, some embodiments include inactivating T cells, preferably graft reactive T cells of the recipient mammal. This can be

accomplished, e.g., by introducing into the recipient mammal an antibody capable of binding to T cells of the recipient mammal. The administration of antibodies, or other treatment to inactivate T cells, can be given prior to introducing the hematopoietic stem cells into the recipient mammal or prior to implanting the graft in the recipient.

Brief Summary Text (49):
Although methods in which a blocker is administered may usually eliminate the need for other preparative steps, some embodiments include the inactivation of thymocytes or T cells, which can be performed prior to hematopoietic stem cell or graft transplantation. In preferred embodiments the method includes diminishing or inhibiting thymocyte or T cell activity, preferably the activity of thymic or lymph node T cells by administering to the recipient a short course of an immunosuppressive agent, e.g., a chemical or drug, e.g., cyclosporine, sufficient to inactivate thymocytes or T cells, preferably thymic or lymph node T cells. The duration of the short course of immunosuppressive agent is: approximately equal to or less than 30, 40, 50, 60, 120, or 365 days; approximately equal to or less than 8-12 days, preferably about 10 days; approximately equal to or less than two, three, four, five, or ten times the 8-12 or 10 day period. The short course can begin: before or at about the time the treatment to induce tolerance is begun, e.g., at about the time stem cells are introduced into the recipient; on the day the treatment to induce tolerance is begun, e.g., on the day stem cells are introduced into the recipient; within 1, 2, 4, 6, 8, 10, or 30 days before or after the treatment to induce tolerance is begun, e.g., within 1, 2, 4, 6, 8, 10, or 30 days before or after stem cells are introduced into the recipient. The short course of an immunosuppressive can be administered in conjunction with an anti-T cell antibody The short course of an immunosuppressive should be sufficient in concentration and duration to inactivate T cells, e.g., thymic or lymph node T cells, which would not be inactivated by antibody-based inactivation of T cells, e.g., inactivation by intravenous administrations of ATG antibody, or similar, preparations.

Brief Summary Text (50):
Although methods in which a blocker is administered may usually eliminate the need for other preparative steps, some embodiments include (optionally): the step of, prior to hematopoietic stem cell transplantation, creating hematopoietic space, e.g., by irradiating the recipient mammal with low dose, e.g., less than 400, preferably less than 300, more preferably less than 200 or 100 cGy, whole body irradiation to deplete or partially deplete the bone marrow of the recipient. As is discussed herein this treatment can be reduced or entirely eliminated. Other methods of creating hematopoietic space, e.g., administering hematopoietic space creating antibodies or drugs, e.g., cyclophosphamide or busulfan, to the recipient, can be used. E.g., hematopoietic space can be formed by administering an inhibitor of cell proliferation, e.g., DSG, or an anti-metabolite, e.g. brequinar, or an anti-T cell antibody, e.g., one or both of an anti-CD4 or anti-CD8 antibody.

Brief Summary Text (51):
In preferred embodiments, the method includes the step of introducing into the recipient a graft obtained from the donor which is obtained from a different organ than the hematopoietic stem cells, e.g., a heart, pancreas, liver, or kidney.

Brief Summary Text (52):
In a particularly preferred embodiment the method includes: administering to a human recipient an inhibitor, e.g., a blocker, the CD28-B7 interaction; introducing, e.g., by intravenous injection, into the recipient mammal, hematopoietic stem cells, e.g., a bone marrow preparation; and implanting the graft in the recipient

Brief Summary Text (55):
In a preferred embodiment the administration of costimulatory blockade, and preferably of any needed irradiation or T cell depletion, is administered within 48, more preferably, within 24, hours of implantation of the graft.

Brief Summary Text (56):
In a preferred embodiment, the graft is a human cadaveric graft.

Brief Summary Text (57):

In another aspect, the invention features a method of promoting acceptance, by a recipient mammal, e.g., a primate, e.g., a human, of a graft from a donor mammal of the same species. The method includes: administering to the recipient, an inhibitor of a costimulatory pathway, e.g., one or both of an inhibitor, e.g., a blocker, of the CD40 ligand-CD40 interaction and an inhibitor, e.g., a blocker, of the CD28-B7 interaction; introducing, e.g., by intravenous injection, into the recipient mammal, hematopoietic stem cells, e.g., a bone marrow preparation, wherein the number of hematopoietic stem cells is sufficient such that mixed hematopoietic chimerism can be induced without whole body irradiation; preferably, implanting the graft in the recipient. The hematopoietic cells are believed to prepare the recipient for the graft that follows, by inducing tolerance at both the B-cell and T-cell levels.

Brief Summary Text (60):
In preferred embodiments CTLA4-Ig and an anti CD40L antibody are administered.

Brief Summary Text (62):
In preferred embodiments, a blocker of the CD40/CD40L interaction, e.g., an anti-CD40L antibody is administered prior to administration of a blocker of the CD28/B7 interaction, e.g., CTLA4/Ig. The CD40/CD40L blocker can be administered on the day donor tissue is introduced and the CD28/B7 blocker administered 2, 3,4 5 or more days later.

Brief Summary Text (69):
Repeated stem cell administration can promote engraftment, mixed chimerism, and preferably long-term deletional tolerance in graft recipients. Thus, the invention also includes methods in which multiple hematopoietic stem cell administrations are provided to a recipient. Multiple administration can substantially reduce or eliminate the need for hematopoietic space-creating irradiation. Administrations can be given prior to, at the time of, or after graft implantation. In preferred embodiments multiple administrations of stem cells are provided prior to the implantation of a graft. Two, three, four, five, or more administrations can be provided. The period between administrations of hematopoietic stem cells can be varied. In preferred embodiments a subsequent administration of hematopoietic stem cell is provided: at least two days, one week, one month, or six months after the previous administration of stem cells; when the recipient begins to show signs of host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain a level of mixed chimerism sufficient to maintain tolerance to donor antigen.

Brief Summary Text (70):
One or more post graft-implantation-administrations of donor stem cells can also be provided to minimize or eliminate the need for irradiation. Post graft administration of hematopoietic stem cells can be provided: at least two days, one week, one month, or six months after the previous administration of stem cells; at least two days, one week, one month, six months, or at any time in the life span of the recipient after the implantation of the graft; when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the host donor specific antibody response, or by a change in the host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a graft.

Brief Summary Text (72):
Although methods in which blockers of both pathways are administered may usually eliminate the need for other preparative steps, some embodiments include inactivating T cells, preferably graft reactive T cells of the recipient mammal. This can be accomplished, e.g., by introducing into the recipient mammal an antibody capable of

binding to T cells of the recipient mammal. The administration of antibodies, or other treatment to inactivate T cells, can be given prior to introducing the hematopoietic stem cells into the recipient mammal or prior to implanting the graft in the recipient.

Brief Summary Text (75):
Although methods in which blockers of both pathways are administered may usually eliminate the need for other preparative steps, some embodiments include the inactivation of thymocytes or T cells, which can be performed prior to hematopoietic stem cell or graft transplantation. In preferred embodiments the method includes diminishing or inhibiting thymocyte or T cell activity, preferably the activity of thymic or lymph node T cells by administering to the recipient a short course of an immunosuppressive agent, e.g., a chemical or drug, e.g., cyclosporine, sufficient to inactivate thymocytes or T cells, preferably thymic or lymph node T cells. The duration of the short course of immunosuppressive agent is: approximately equal to or less than 30, 40, 50, 60, 120, or 365 days; approximately equal to or less than 8-12 days, preferably about 10 days; approximately equal to or less than two, three, four, five, or ten times the 8-12 or 10 day period. The short course can begin: before or at about the time the treatment to induce tolerance is begun, e.g., at about the time stem cells are introduced into the recipient; on the day the treatment to induce tolerance is begun, e.g., on the day stem cells are introduced into the recipient; within 1, 2, 4, 6, 8, 10, or 30 days before or after the treatment to induce tolerance is begun, e.g., within 1, 2, 4, 6, 8, 10, or 30 days before or after stem cells are introduced into the recipient. The short course of an immunosuppressive can be administered in conjunction with an anti-T cell antibody The short course of an immunosuppressive should be sufficient in concentration and duration to inactivate T cells, e.g., thymic or lymph node T cells, which would not be inactivated by antibody-based inactivation of T cells, e.g., inactivation by intravenous administrations of ATG antibody, or similar, preparations.

Brief Summary Text (76):
Although methods in which blockers of both pathways are administered may usually eliminate the need for other preparative steps, some embodiments include (optionally): the step of, prior to hematopoietic stem cell transplantation, creating hematopoietic space, e.g., by irradiating the recipient mammal with low dose, e.g., less than 400, preferably less than 300, more preferably less than 200 or 100 cGy, whole body irradiation to deplete or partially deplete the bone marrow of the recipient. As is discussed herein this treatment can be reduced or entirely eliminated. Other methods of creating hematopoietic space, e.g., administering hematopoietic space creating antibodies or drugs, e.g., cyclophosphamide or busulfan, to the recipient, can be used. E.g., hematopoietic space can be formed by administering an inhibitor of cell proliferation, e.g., DSG, or an anti-metabolite, e.g. brequinar, or an anti-T cell antibody, e.g., one or both of an anti-CD4 or anti-CD8 antibody.

Brief Summary Text (77):
Other preferred embodiments include those in which: the same mammal is the donor of one or both the graft and the hematopoietic cells; and the antibody is an anti-human thymocyte polyclonal anti-serum, obtained, e.g., from a horse or pig.

Brief Summary Text (78):
In preferred embodiments, the method includes the step of introducing into a human recipient, a graft obtained from the donor which is obtained from a different organ than the hematopoietic stem cells, e.g., a heart, pancreas, liver, or kidney.

Brief Summary Text (79):
In a particularly preferred embodiment the method includes: administering to a human recipient, a blocker of the CD40 ligand-CD40 interaction (optionally, a blocker of the CD28-B7 interaction can also be administered); introducing, e.g., by intravenous injection, into the recipient mammal, hematopoietic stem cells, e.g., a bone marrow preparation; and implanting the graft in the recipient.

Brief Summary Text (82):
In a preferred embodiment the administration of costimulatory blockade, and preferably of any needed irradiation or T cell depletion, is administered within 48, more preferably, within 24, hours of implantation of the graft.

Brief Summary Text (83):
In another aspect, the invention features, a method of promoting acceptance by a recipient mammal, e.g., a primate, e.g., a human, of a graft from a donor mammal. The method includes: administering to the recipient, an inhibitor, e.g., a blocker, of a costimulatory pathway, (e.g., one or both of, an inhibitor, e.g., a blocker, of the CD40 ligand-CD40 interaction and an inhibitor, e.g., a blocker, of the CD28-B7 interaction); prior to or simultaneous with transplantation of the graft, introducing into the recipient mammal, donor thymic tissue, e.g., thymic epithelium, preferably fetal or neonatal thymic tissue; and (optionally) implanting the graft in the recipient. The thymic tissue prepares the recipient for the graft that follows, by inducing immunological tolerance at the T-cell level.

Brief Summary Text (89):
In preferred embodiments, a blocker of the CD40/CD40L interaction, e.g., an anti-CD40L antibody, is administered prior to administration of a blocker of the CD28/B7 interaction, e.g., CTLA4/Ig. The CD40/CD40L blocker can be administered on the day donor tissue is introduced and the CD28B7 blocker administered 2, 3, 4, 5 or more days later.

Brief Summary Text (91):
The recipient mammal can be, by way of example, a human. The donor mammal can be, by way of example, a swine, e.g., a miniature swine. The graft is preferably from a discordant species. The graft preferably expresses a major histocompatibility complex (MHC) antigen, preferably a class II antigen. In particularly preferred embodiments the recipient is a primate, e.g., a human, and the donor is a swine, e.g., a miniature swine. antibodies.

Brief Summary Text (96):
Although methods in which blockers of both pathways are administered may usually minimize or eliminate the need for other preparative steps, some embodiments include inactivating natural killer cells, preferably graft reactive or xenoreactive, e.g., swine reactive, NK cells, of the recipient mammal. This can be accomplished, e.g., by introducing into the recipient mammal an antibody capable of binding to natural killer cells of the recipient mammal, e.g., an anti-CD2 antibody, e.g., MEDI-507. The administration of antibodies, or other treatment to inactivate natural killer cells, can be given prior to introducing the thymic tissue into the recipient mammal or prior to implanting the graft in the recipient. This antibody can be the same or different from an antibody used to inactivate T cells.

Brief Summary Text (97):
Although methods in which blockers of both pathways are administered may usually minimize or eliminate the need for other preparative steps, some embodiments include inactivating T cells, preferably graft reactive or xenoreactive, e.g., swine reactive, T cells of the recipient mammal. This can be accomplished, e.g., by introducing into the recipient mammal an antibody capable of binding to T cells of the recipient mammal. The administration of antibodies, or other treatment to inactivate T cells, can be given prior to introducing the thymic tissue into the recipient mammal or prior to implanting the graft in the recipient. This antibody can be the same or different from an antibody used to inactivate natural killer cells.

Brief Summary Text (98):
One source of anti-NK antibody is anti-human thymocyte polyclonal anti-serum. Preferably, a second anti-mature T cell antibody can be administered as well, which lyses T cells as well as NK cells. Lysing T cells is advantageous for both thymic tissue and graft survival. Anti-T cell antibodies are present, along with anti-NK antibodies, in anti-thymocyte anti-serum. Repeated doses of antibodies, e.g., anti-NK or anti-T cell antibodies, may be preferable. Monoclonal preparations can be used in the methods of the invention.

Brief Summary Text (99):
Although methods in which blockers of both pathways are administered may usually minimize or eliminate the need for other preparative steps, some embodiments include (optionally): the step of, prior to hematopoietic stem cell transplantation, creating hematopoietic space, e.g., by irradiating the recipient mammal with low dose, e.g.,

less than 400, preferably less than 300, more preferably less than 200 or 100 cGy, whole body irradiation to deplete or partially deplete the bone marrow of the recipient. As is discussed herein this treatment can be reduced or entirely eliminated. Other methods of creating hematopoietic space, e.g., administering hematopoietic space creating antibodies or drugs, e.g., cyclophosphamide or busulfan, to the recipient, can be used. E.g., hematopoietic space can be formed by administering an inhibitor of cell proliferation, e.g., DSG, or an anti-metabolite, e.g. brequinar, or an anti-T cell antibody, e.g., one or both of an anti-CD4 or anti-CD8 antibody.

Brief Summary Text (100):
Other preferred embodiments include: the step of, preferably prior to hematopoietic or thymic tissue transplantation, depleting natural antibodies from the blood of the recipient mammal. Depletion can be achieved, by way of example, by contacting the recipients blood with an epitope which absorbs preformed anti-donor antibody. The epitope can be coupled to an insoluble substrate and provided, e.g., as an affinity column. E.g., an .alpha.1-3 galactose linkage epitope-affinity matrix, e.g., matrix bound linear B type VI carbohydrate, can be used to deplete natural antibodies. Depletion can also be achieved by hemoperfusing an organ, e.g., a liver or a kidney, obtained from a mammal of the donor species. (In organ hemoperfusion antibodies in the blood bind to antigens on the cell surfaces of the organ and are thus removed from the blood.)

Brief Summary Text (101):
Other preferred embodiments include those in which: the same mammal of the second species is the donor of one or both the graft and the hematopoietic cells; and the antibody is an anti-human thymocyte polyclonal anti-serum, obtained, e.g., from a horse or pig.

Brief Summary Text (102):
In preferred embodiments, the method includes the step of introducing into the recipient a graft obtained from the donor which is obtained from a different organ than the hematopoietic stem cells, e.g., a heart, pancreas, liver, or kidney.

Brief Summary Text (104):
In preferred embodiments the method further includes the step of identifying a host or recipient which is in need of a graft.

Brief Summary Text (106):
In a preferred embodiment the administration of costimulatory blockade, and preferably of any needed irradiation or T cell depletion, is administered within 48, more preferably, within 24, hours of implantation of the graft.

Brief Summary Text (109):
"Discordant species combination", as used herein, refers to two species in which hyperacute rejection occurs when a graft is grafted from one to the other. Generally, discordant species are from different orders, while non-discordant species are from the same order. For example, rats and mice are non-discordant concordant species. Concordant species combinations do not exhibit hyperacute rejection.

Brief Summary Text (110):
"Graft", as used herein, refers to a body part, organ, tissue, or cells. Organs such as liver, kidney, heart or lung, or other body parts, such as bone or skeletal matrix, tissue, such as skin, intestines, endocrine glands, or progenitor stem cells of various types, are all examples of grafts.

Brief Summary Text (112):
"Immunosuppressive agent capable of inactivating thymic or lymph node T cells", as used herein, is an agent, e.g., a chemical agent, e.g., a drug, which, when administered at an appropriate dosage, results in the inactivation of thymic or lymph node T cells. Examples of such agents are cyclosporine, FK-506, and rapamycin. Anti-T cell antibodies can also be used. An agent should be administered in sufficient dose to result in significant inactivation of thymic or lymph node T cells which are not inactivated by administration of an anti-T cell antibody, e.g., an anti-ATG preparation. Putative agents, and useful concentrations thereof, can be prescreened by

in vitro or in vivo tests, e.g., by administering the putative agent to a test animal, removing a sample of thymus or lymph node tissue, and testing for the presence of active T cells in an in vitro or in vivo assay. Such prescreened putative agents can then be further tested in transplant assays.

Brief Summary Text (120):
"Promoting acceptance of a graft" as used herein, refers to any of increasing the time a graft is accepted or is functional or decreasing the recipients immune response to the graft, e.g., by the induction of tolerance.

Brief Summary Text (121):
"Tolerance", as used herein, refers to an inhibition of a graft recipient's immune response which would otherwise occur, e.g., in response to the introduction of a nonself MHC antigen into the recipient. Tolerance can involve humoral, cellular, or both humoral and cellular responses. Tolerance, as used herein, refers not only to complete immunologic tolerance to an antigen, but to partial immunologic tolerance, i.e., a degree of tolerance to an antigen which is greater than what would be seen if a method of the invention were not employed. Tolerance, as used herein, refers to a donor antigen-specific inhibition of the immune system as opposed to the broad spectrum inhibition of the immune system seen with immunosuppressants.

Brief Summary Text (126):
The invention provides a reliable, non-toxic method of inducing transplantation tolerance. It minimizes the problems of chronic organ graft rejection and immunosuppression-related toxicity. BMT with CTLA4Ig plus MR1 specifically minimizes or eliminates donor-reactive T cells, while avoiding the non-specific depletion or suppression of T cells, which is a component of clinically available immunosuppressive strategies, and can lead to severe complications. This treatment protocol is suitable for both cadaveric and living-related organ transplantation, as it allows the reliable induction of deletional tolerance with a non-toxic conditioning regimen beginning on the day of transplantation. Since the peripheral T cell repertoire is not globally depleted by the conditioning and only a low, minimally myelosuppressive dose of whole body irradiation is given, the clinical usefulness of this approach is extraordinarily high.

Drawing Description Text (5):
FIG. 2 is a plot of graft survival versus day past grafting. Permanent survival of donor-specific skin grafts in chimeras prepared with 3 Gy WBI and allogeneic (B10.A) BMC and treatment with MR1 plus CTLA4Ig was seen. Combined results from two experiments are shown. Recipients were grafted with donor-specific (B10.A) and third party (A.SW) skin grafts at 3, 6 or 10 weeks after BMT. Mice receiving the full treatment of BMT and MR1 plus CTLA4Ig accepted donor skin grafts (B) permanently (12 out of 14), with the exception of two animals that rejected their grafts at days 57 and 76, respectively. Nine grafts have been accepted in perfect condition for more than 110 days, and 5 grafts for more than 140 days. Third-party skin grafts (B) were rejected in the expected time-frame (MST)=10d). MR1 alone (A) led to prolongation of donor-specific skin graft survival (MST=42d), but only 2 out of 9 grafts survived more than 100 days. CTLA4Ig alone (A) failed to improve skin graft survival (n=7, MST=10d). Control mice treated with 3 Gy WBI plus BMC (n=4) and mice receiving 3 Gy WBI and MR1 plus CTLA4Ig alone (without BMT, not shown) rejected donor skin within 2 weeks. A control group prepared with TCD mAbs on d-5 and d-1 plus BMT (+3 Gy WBI) (n=5), accepted donor skin grafts permanently in 60%. Third party grafts were rejected within 2 weeks in all groups.

Detailed Description Text (1):
SOURCES OF CELLS FOR ALLOGENEIC STEM CELL TRANSPLANTATION

Detailed Description Text (3):
Sources of Cells for Xenogeneic Stem Cell Transplantation

Detailed Description Text (10):
To remove natural antibodies from the recipient's circulation prior to transplantation, on day 0 an operative absorption of natural antibodies (nAB) is performed, using an .alpha.1-3 galactose linkage epitope-affinity matrix.

Detailed Description Text (12):
Swine donor hematopoietic cells, e.g., bone marrow cells, are administered by intravenous injection. Bone marrow is harvested and injected intravenously as previously described (Pennington et al., 1988, Transplantation 45:21-26). 7.5.times.10.sup.8 /kg bone marrow cells are typically administered in regimens which include WBI. Initial trials to determine an appropriate number of cells to be administered in a regimen which lacks WBI should begin with a range of doses from several times to 20 times that number. Multiple administrations are desirable in the higher end of the dosage range. Swine cytokines can be administered to promote engraftment.

Detailed Description Text (13):
To follow chimerism, two color flow cytometry can be used. This assay uses monoclonal antibodies to distinguish between donor class I major histocompatibility antigens and leukocyte common antigens versus recipient class I major histocompatibility antigens. Alternatively chimerism can be followed by PCR. Should natural antibodies be found to recur before tolerance is induced, and should these antibodies cause damage to the donor tissue, the protocol can be modified to permit sufficient time following BMT for humoral tolerance to be established prior to organ grafting. Tolerance to donor antigen can be followed by standard methods, e.g., by MLR assays.

Detailed Description Text (14):
The Induction of Tolerance with Bone Marrow Transplantation

Detailed Description Text (15):
The following procedure was designed to lengthen the time an implanted organ (a xenograft) survives in a xenogeneic host prior to rejection. The organ can be any organ, e.g., a liver, a kidney, a pancreas, or a heart. The method main strategies include one or more of the following: administration of inhibitors of the CD40 ligand-CD40 and the CD28-B7 interaction and the elimination of natural antibodies, e.g., by contacting the recipient's blood with epitopes which react with donor-reactive natural antibody; transplantation of tolerance-inducing stem cells, e.g., bone marrow stem cells, optionally, the implantation of donor stromal tissue or administration of donor cytokines. The combination of a sufficiently large number of administered donor stem cells in combination with inhibition of the two pathways significantly reduces or eliminates the need for whole body irradiation, thymic irradiation, and anti-T cell antibodies. The method includes any or all of these steps. Preferably they are carried out in the following sequence.

Detailed Description Text (16):
First, a preparation of anti-CD40 ligand monoclonal antibody and CTLA4-1gG fusion protein are administered to the subject.

Detailed Description Text (18):
Second, natural antibodies are absorbed from the recipient's blood by hemoperfusion. Pre-formed natural antibodies (nAB) are the primary agents of graft rejection. Natural antibodies bind to xenogeneic endothelial cells. These antibodies are independent of any known previous exposure to antigens of the xenogeneic donor. The mechanism by which newly developing B cells are tolerized is unknown. An .alpha.1-3 galactose linkage epitope-affinity matrix, e.g., in the form of an affinity column, e.g., matrix bound linear B type VI carbohydrate, is useful for removing anti-swine antibodies from the recipient's blood.

Detailed Description Text (20):
Fourth, bone marrow cells (BMC), or another source of hematopoietic stem cells, e.g., a fetal liver suspension, of the donor are injected into the recipient. Donor BMC home to appropriate sites of the recipient and grow contiguously with remaining host cells and proliferate, forming a mixed chimeric lymphohematopoietic population. By this process, newly forming and pre-existing B cells are exposed to donor antigens, so that the transplant will be recognized as self. Tolerance to the donor is also observed at the T cell level in animals in which hematopoietic stem cell, e.g., BMC, engraftment has been achieved. When an organ graft is placed in such a recipient several months after bone marrow chimerism has been induced, natural antibody against the donor will have disappeared, and the graft should be accepted by both the humoral and the cellular arms of the immune system. This approach has the added advantage of

permitting organ transplantation to be performed sufficiently long following transplant of hematopoietic cells, e.g., BMT, e.g., a fetal liver suspension, that normal health and immunocompetence will have been restored at the time of organ transplantation. The use of xenogeneic donors allows the possibility of using bone marrow cells and organs from the same animal, or from genetically matched animals.

Detailed Description Text (23):
While any of these procedures may aid the survival of an implanted organ, best results are achieved when all steps are used in combination. Methods of the invention can be used to confer tolerance to allogeneic grafts, e.g., wherein both the graft donor and the recipient are humans, and to xenogeneic grafts, e.g., wherein the graft donor is a nonhuman animal, e.g., a swine, e.g., a miniature swine, and the graft recipient is a primate, e.g., a human.

Detailed Description Text (24):
The approaches described above are designed to synergistically prevent the problem of transplant rejection.

Detailed Description Text (26):
The method of introducing bone marrow cells may be altered, particularly by (1) increasing the time interval between injecting hematopoietic stem cells and implanting the graft; (2) increasing the amount of hematopoietic stem cells injected; (3) varying the number of hematopoietic stem cell injections; (4) varying the method of delivery of hematopoietic stem cells; (5) varying the tissue source of hematopoietic stem cells, e.g., a fetal liver cell suspension may be used; or (6) varying the donor source of hematopoietic stem cells. Although hematopoietic stem cells derived from the graft donor are preferable, hematopoietic stem cells may be obtained from other individuals or species, or from genetically-engineered inbred donor strains, or from in vitro cell culture.

Detailed Description Text (27):
Methods of preparing the recipient for transplant of hematopoietic stem cells may be varied. For instance, recipient may undergo a splenectomy. The latter would preferably be administered prior to the non-myeloablative regimen, e.g., at day -14.

Detailed Description Text (30):
Stromal tissue introduced prior to hematopoietic cell transplant, e.g., BMT, may be varied by: (1) administering the fetal liver and thymus tissue as a fluid cell suspension; (2) administering fetal liver or thymus stromal tissue but not both; (3) placing a stromal implant into other encapsulated, well-vascularized sites, or (4) using adult thymus or fetal spleen as a source of stromal tissue.

Detailed Description Text (32):
In this animal trial, the treatment of mice with single injections of an anti-CD40 ligand-antibody and CTLA4Ig, a low dose (3 Gy) of whole body irradiation, plus fully MHC-mismatched allogeneic bone marrow transplantation reliably induced high levels (>40%) of stable (>8 months) multi-lineage donor hematopoiesis. Chimeric mice permanently accepted donor skin grafts (>100 days), and rapidly rejected third party grafts. Progressive deletion of donor-reactive host T cells occurred among peripheral CD4+ lymphocytes, beginning as early as one week after bone marrow transplantation. Early deletion of peripheral donor-reactive host CD4 cells also occurred in thymectomized, similarly-treated marrow recipients, demonstrating a role for peripheral clonal deletion of donor-reactive T cells after allogeneic bone marrow transplantation in the presence of costimulatory blockade. Central intrathymic deletion of newly-developing T cells ensued after donor stem cell engraftment had occurred.

Detailed Description Text (33):
Solid organ (skin) grafting was not required in the induction phase of tolerance in model. Instead, the permanent engraftment of donor hematopoietic cells ensured the tolerization of pre-existing host T cells and of T cells that developed subsequent to the disappearance of the costimulatory blocking agents from the circulation. This later tolerance occurred through intrathymic deletional mechanisms (FIG. 4), presumably as a consequence of the presence of donor-derived APC in the thymus, as has been demonstrated in long-term mixed chimeras prepared with other regimes that involve

initial depletion of the T cell repertoire with mAbs. The work described herein shows that costimulatory blockade leads to peripheral deletion of donor-reactive T cells, then allows the engraftment of fully MHC-mismatched, allogeneic pluripotent stem cells, which induce central tolerance among T cells that subsequently develop in the thymus.

Detailed Description Text (37):
Conditioning and Bone Marrow Transplantation

Detailed Description Text (40):
Flow-cytometric analysis (FCM) of multi-lineage chimerism was performed as previously described in Tomita, Y., D. H. Sachs, A. Khan, and M. Sykes. 1996,Transplantation 61:469-477. Briefly, forward angle and 90 degree light scatter properties were used to distinguish lymphocytes, monocytes and granulocytes in peripheral white blood cells. Two-color FCM was utilized to distinguish donor and host cells of particular lineages, and the percentage of donor cells was calculated as described in Tomita, Y., D. H. Sachs, A. Khan, and M. Sykes. 1996, Transplantation 61:469-477, by subtracting control staining from quadrants containing donor and host cells expressing a particular lineage marker, and by dividing the net percentage of donor cells by the total net percentage of donor plus host cells of that lineage. Dead cells were excluded using propidium iodide staining. Non-specific FcgR binding was blocked by anti-mouse FcgR mAb 2.4G2, Unkeless, J. C. 1979, J.Exp.Med. 150:580-596. Fluorescin isocyanate (FITC)-conjugated mAbs included anti-CD4, anti-CD8, anti-B220 (all purchased from PharMingen, San Diego, Calif.) and anti-MAC1 (Caltag, San Francisco, Calif.). Negative control mAb HOPC1-FITC, with no reactivity to mouse cells, was prepared in our laboratory. Biotinylated anti-H-2Dd mAb 34-2-12 and control mAb HOPC1 were developed with phycoerythrin-streptavidin (PEA).

Detailed Description Text (43):
Skin Grafting

Detailed Description Text (44):
Full thickness tail skin from B10.A (donor-specific) and fully MHC-mismatched A.SW (third party) mice was grafted onto the lateral thoracic wall, secured with 5-0 silk sutures and bandaids and followed by visual and tactile inspections daily for three weeks, then at least every week thereafter. Grafts were defined as rejected when less than 10% of the graft remained viable.

Detailed Description Text (48):
Donor-specific Skin Graft Tolerance in Chimeras Prepared with CTLA4Ig Plus MR1

Detailed Description Text (49):
Primary skin grafting is considered the most stringent test of transplantation tolerance. Therefore, donor (B10.A) and third party (A.SW) full-thickness tail skin were grafted onto recipients at various time points after BMT. Mice that received both CTLA4Ig and MR1, plus 3 Gy WBI and BMT, permanently accepted donor skin grafts placed 3, 6 or 10 weeks after BMT (FIG. 2B), with the exception of two animals that rejected their grafts 57 and 76 days after graft placement, respectively. Third party grafts were readily rejected (median survival time (MST)=10d), demonstrating the donor specificity of the tolerance induced. This skin graft survival compares favorably even to the control animals that were conditioned with T cell-depleting antibodies, in which only 60% of donor skin grafts survived more than 100 days (FIG. 2A). Mice treated with MR1 alone in addition to 3 Gy WBI and BMT demonstrated prolongation of donor skin graft survival (MST=42d) (FIG. 2A). However, only 2 out of 9 grafts were accepted for 100 days. In contrast, in this particular protocol, mice receiving BMT following 3 Gy WBI and CTLA4Ig alone did not show prolonged survival of donor skin grafts (MST=10d), consistent with the absence of chimerism.

Detailed Description Text (50):
These results demonstrate the presence of donor-specific tolerance across a full MHC barrier in chimeras prepared with MR1 plus CTLA4Ig. The ability of mixed chimeras prepared with MR1 and CTLA4Ig to rapidly reject third party skin grafts is evidence for their immunocompetence.

Detailed Description Text (53):

Partial deletion of V.beta.5+ and V.beta.11+ peripheral CD4 T cells was observed as early as one week after BMT in mice receiving 3 Gy WBI followed by MR1 plus CTLA4Ig (FIG. 3). The deletion became progressively more complete over the ensuing weeks, and reached similar levels to those in chimeras prepared with T cell depletion (not shown). Deletion of these V.beta.5+ and V.beta.11+ cells was sustained throughout the follow-up period (>6 months in the first experiment for which chimerism data are shown in FIG. 1). Percentages of V.beta.8- bearing CD4 cells, which do not recognize superantigens on the donor or host, were not reduced at any time point, ruling out a non-specific deletional process. Mice treated with BMT (plus 3 Gy WBI) and MR1 alone showed early partial deletion of V.beta.5 and V.beta.11, which was only transient in the experiment shown (FIG. 3). However, in the experiment shown in FIG. 1, deletion was still observed at later time points for the group receiving BMT (plus 3 Gy WBI) and MR1 alone, which correlated with the higher initial levels of chimerism observed for this group in this experiment. Control animals receiving 3 Gy WBI plus BMT alone or BMT (plus 3 Gy WBI) with CTLA4Ig failed to show any V.beta.5 or V.beta.11 deletion. As expected, deletion of V.beta.5 and V.beta.11 did not occur in control animals receiving WBI and MR1 plus CTLA4Ig without BMT (not shown). Down-regulation of the level of TCR expression instead of deletion seems an unlikely explanation for the reduction in V.beta.5+ and V.beta.11+ CD4 T cells in chimeras, since the intensity of the V.beta.5 and V.beta.11 staining on the cells remaining in the blood at one and three weeks post-BMT was similar to that in non-transplanted controls (data not shown). Thus, no evidence for TCR down-modulation was observed.

Detailed Description Text (58):
Peripheral deletion has been shown to be one consequence of powerful T cell responses in vivo, but it has only been reported following marked expansion of antigen-recognizing cells. Although one week post-transplant was the earliest time point at which donor-reactive host T cells were examined, evidence of such initial expansion was not seen. More recently, in vitro evidence has demonstrated that costimulatory signals play a prominent role in preventing apoptotic cell death following TCR engagement. However, apoptosis induced in vivo by antigen encountered in the presence of costimulatory blockade has not been described.

Detailed Description Text (64):
C57BL/6 mice received depleting anti-CD4 and anti-CD8 mAbs on day -5, 3 Gy whole body irradiation (WBI, day 0), and 15.times.10.sup.6 fully MHC-mismatched, B10.A bone marrow cells (BMC). In addition, hosts were injected with an anti-CD154 mAb (day 0) and/or CTLA4Ig (day +2). Chimerism in peripheral blood was followed by FACS analysis, and tolerance was assessed by skin grafting, and also by MLR and CML assays. The frequency of certain V.beta. families was determined by FACS to assess deletion of donor-reactive T cells.

Detailed Description Text (65):
Chimerism was transient and tolerance was not present in animals receiving TCD mAbs on day -5 without costimulatory blockade. The addition of anti-CD154 mAb (CD154 is also called CD40 ligand and gp39) and CTLA4Ig, alone or in combination, reliably permitted induction of high levels of stable (>6 months) multilineage chimerism, with specific tolerance to skin grafts and donor antigens by MLR and CML assays. Long-term chimeras showed deletion of donor-reactive CD4.sup.+ PBL, splenocytes and mature thymocytes. Administration of TCD mAbs only one day prior to bone marrow transplantation (BMT) plus anti-CD154 mAb also allowed induction of permanent chimerism and tolerance.

Detailed Description Text (66):
Thus, one injection of anti-CD154 mAb or CTLA4Ig overcomes the need for TI or prolonged host TCD for the induction of mixed chimerism and deletional tolerance and thus further decreases the toxicity of this protocol. Achievement of tolerance with conditioning given over 24 hours makes this approach even more useful for cadaveric organ transplantation.

Detailed Description Text (70):
Conditioning and Bone Marrow Transplantation (BMT)

Detailed Description Text (83):
Skin Grafting

Detailed Description Text (84):
Full thickness tail skin from B10. A (donor-specific) and fully MHC-mismatched A. SW (third party) mice was grafted onto the lateral thoracic wall 5 to 10 weeks after BMT, secured with 5-10 silk sutures and bandaids which were removed one week later. Grafts were then followed by daily visual and tactile inspections for the first three weeks, and at least weekly thereafter. Grafts were defined as rejected when less than 10% of the graft remained viable.

Detailed Description Text (89):
One group of recipients (Group A) was treated with the previously described regimen (Tomita et al., 1996, Transplantation 61: 469.) of two doses of TCD mAbs on days -5 and -1. Three of five of these recipients developed long-lasting multilineage chimerism, with the mean percentage of donor representation among CD4 and CD8 cells being lower than donor chimerism among B cells and myeloid lineages. The remaining two mice initially developed high levels of B cell and myeloid chimerism, but lower levels of T cell chimerism (apparent at 7 weeks), and chimerism in all lineages began to decline soon after BMT.

Detailed Description Text (102):
Skin Graft Survival

Detailed Description Text (103):
To determine whether donor-specific tolerance was induced, skin grafting, which is the most stringent test for transplantation tolerance, was performed. Donor and third-party skin was grafted ten weeks, seven weeks (second experiment) or five weeks (third experiment) after BMT. In the group receiving two doses of TCD mAbs (Group A) the three successful chimeras accepted donor skin grafts permanently (>130 days), while all mice prepared with TCD mAbs only on day -5 without further treatment (Group B) rejected their grafts within 15 days, consistent with the absence of long-term chimerism.

Detailed Description Text (104):
In the group of recipients treated with TCD mAbs on day -5 plus CTLA4Ig alone (Group C), donor grafts were accepted permanently by the three successful chimeras. One of the two mice that failed to develop high levels of multilineage chimerism. rejected its donor graft on day 9, and the second one died with its graft still in good condition 16 days after BMT. All animals receiving anti-CD154 mAb alone (Group D) or anti-CD154 mAb plus CTLA4Ig (Group E) accepted their donor grafts permanently. Long-term surviving grafts remained in perfect condition with a follow-up of up to 160 days.

Detailed Description Text (105):
Control mice receiving conditioning without BMT, or TCD mAbs on day -5 plus control antibodies, promptly rejected their donor grafts. All groups uniformly rejected third party grafts within two weeks, indicating that the induced tolerance was specific and that the chimeras were immunocompetent.

Detailed Description Text (106):
The second experiment showed similar donor-specific skin graft acceptance in mice receiving TCD and costimulatory blockade. The chimeras in the third experiment receiving TCD mAbs on day -1 and anti-CD154 mAb accepted their donor grafts (>40 days), while rejecting third party grafts promptly.

Detailed Description Text (108):
To further evaluate the development of tolerance, MLR and CML assays were performed at the time of sacrifice in the first experiment. Four out of six tested chimeras from Groups C, D and E showed unresponsiveness toward donor antigens, while maintaining reactivity to third-party antigens (stimulation index >1.9). The chimera from Group A, one of two chimeras each from Groups C and E, the mouse from Group B, and a control receiving the conditioning without BMT, were globally hyporesponsive. Results from the CML assays showed a similar pattern as seen with MLR reactivity (Table 2). One chimera from each of Groups C and D demonstrated effective killing of third-party targets while not killing donor targets. The remaining animals, including the control mouse that did not receive BMT, showed general hyporesponsiveness, even though immunocompetence was demonstrated in vivo by the ability to promptly reject third

party skin grafts. Although the senescence of the mice may have caused the generalized in vitro hyporesponsiveness observed in some cases, these MLR and CML studies overall provide further evidence for the presence of donor-specific tolerance in chimeras prepared with costimulatory blockade.

Detailed Description Text (113):
The present studies demonstrate that T cell costimulatory blockade is a potent. Both anti-CD154 mAb and CTLA4Ig were effective as single agents in replacing thymic irradiation or the repeated administration of TCD mAbs. The clinical relevance of this newly developed regimen for tolerance induction is further increased by the ability to begin the conditioning treatment only 24 hours before BMT, making it applicable to cadaveric organ transplantation.

Detailed Description Text (122):
Both peripheral blood samples and apheresis products were assayed for the presence of LTC-CFC which are thought to represent the more primitive pluripotent progenitors (stem cells). Analysis showed that LTC-CFC were not detected on days 0, 1 and 2, but they were observed by day 5 (not assayed on days 3-4) in the peripheral blood (FIG. 9a). They remained consistently detectable during the course of cytokine treatment. (Samples from days 9, 10 and 19 were also not assayed.) Similarly, all apheresis products contained detectable levels of LTC-CFC (FIG. 9b). These results show that the mobilization protocol used provides a sustained mobilization of early progenitors, presumably stem cells into the peripheral blood. In vivo studies have confirmed that PBPC collected according to this protocol can provide long-term reconstitution of multiple hematopoietic lineages when transplanted into allogeneic miniature swine.

Detailed Description Text (123):
A cytokine regimen for mobilizing pig progenitors into the peripheral blood has been defined which produces large numbers of PBPC for transplant. The success of these experiments has provided sufficent donor preparations for high does allogeneic studies to demonstrate mixed chimerism and specific immune tolerance in the absence of WBI and to allow high does pig to primate studies for the optimization of xenogeneic engraftrment.

Detailed Description Text (126):
The following is a model protocol for pig to non-human primate transplant. The treatment protocol used in this study consists of splenectomy, non-myeloablative whole body irradiation (2.times.150 cGy) on days -6 and -5, 700 cGy of thymic irradiation on day -1, treatment with ATG (3.times.50 mg/kg/day on days-3, -2, and -1), myeophenoplate mofetil (MMF) 250 mg/kg/day on days -8 through day 0 and 175 mg/kg/day on days (0-20, cobra venom factor (CVF), pig cytokines (continuous intravenous administration of porcine IL-3 (200 .mu.g/kg) and 1000 .mu.g/kg porcine stem cell factor days 0-14) and high doses of pig peripheral blood progenitor cells (PBPC) (3 doses each of 10.times.10.backslash.s(10) PBPC on days 0, 1, and 2), two doses of anti-Cda=154 mAb (20 mg/kg) on days 0 and 2, and CsA.

Detailed Description Text (127):
CVF is included because it depletes complement, thus inhibiting complement-mediated cytotoxicity which is known to be a major contributor to hyperacute rejection. CVF is administered at the beginning of the treatment at 0.25 mg/kg and when complement levels are higher than 5%-10% of the initial activity. Prostacycline, heparin and methylprednisoione may be given as protective therapy to diminish thrombotic events. Day 0 is defined as the day of transplantation of PBPC and organ graft. Evidence for engraftment of the pig cells in the baboon is provided by demonstration of circulating pig cells in the peripheral blood either by flow cytometry or by PCR analysis.

Detailed Description Text (128):
Ten days prior to transplantation (day -2) adult baboons are set up with lines by exposure and cannulation of the aorta and vena cava using silastic shunts to create a loop. The lines run through a Synsorb 90 Gal .alpha.1,3 Gal affinity column, (Alberta Research Council, Edmonton, Alberta, Canada) prepared according to the manufacturer's directions. During this procedure, the baboon is anesthetized with halothane and maintained by general endotracheal intubation anesthesia with monitoring of blood pressure, blood oxygen, blood gases and pH. The baboon's blood is perfused through the column for 60 minutes. The efficacy of this technique for removing antibodies specific

for Gal.alpha.1,3Gal epitope is measured by flow cytometry. On the same day, treatment with cyclosporine at a blood level of 1600 ng/ml is initiated and maintained thereafter. The column perfusion is repeated at day -1, and day 0. Kidney xenografts are placed intraperitoneally. Renal vessels are anastomosed to the aorta and infrarenal ven cava at the sites previously cannulated during hemoperfusion. The urinary tract is reconstructed by extravesical ureteroneccystomy, and native ureters are ligated at the time of transplantation. Recipients are supported perioperatively with washed nonhuman packed red cells and platelets are required to maintain hematocrit above 20% and platelets above 20,000/mm.backslash.s(3).

Detailed Description Text (130):
The methods described herein for inducing tolerance to, or promoting the acceptance of, an allogeneic antigen or allogeneic graft can be used where, as between the donor and ecipient, there is any degree of mismatch at MHC loci or other loci which influence graft ejection. There can be a mismatch at at least one MHC locus or at at least one other locus hat mediates recognition and rejection, e.g., a minor antigen locus. With respect to class I nd class II MHC loci, the donor and recipient can be: matched at class I and mismatched at class II; mismatched at class I and matched at class II; mismatched at class I and mismatched at class II; matched at class I, matched at class II. In any of these combinations other loci which control recognition and rejection, e.g., minor antigen loci, can be matched or mismatched. As stated above, it is preferable that there is mismatch at least one locus. Mismatched at MHC class I means mismatched for one or more MHC class I loci, e.g., in the case of humans, mismatched at one or more of HLA-A, HLA-B, or HLA-C, or in the case of swine, mismatch at one or more SLA class I loci, e.g., the swine A or B loci. Mismatched at MHC class II means mismatched at one or more MHC class II loci, e.g., in the case of humans, mismatched at one or more of a DP .alpha., a DP.beta., a DQ .alpha., a DQ .beta., a DR .alpha., or a DR .beta., or in the case of swine, mismatch at one or SLA class II loci, e.g., mismatch at DQ .alpha. or .beta., or DR .alpha. or .beta..

Detailed Description Text (131):
The methods described herein for inducing tolerance to an allogeneic antigen or allogeneic graft can be used where, as between the donor and recipient, there is any degree of reactivity in a mixed lymphocyte assay, e.g., wherein there is no, low, intermediate, or high mixed lymphocyte reactivity between the donor and the recipient. In preferred embodiments mixed lymphocyte reactivity is used to define mismatch for class II, and the invention includes methods for performing allogeneic grafts between individuals with any degree of mismatch at class II as defined by a mixed lymphocyte assay. Serological tests can be used to determine mismatch at class I or II loci and the invention includes methods for performing allogeneic grafts between individuals with any degree of mismatch at class I and or II as measured with serological methods. In a preferred embodiment, the invention features methods for performing allogeneic grafts between individuals which, as determined by serological and or mixed lymphocyte reactivity assay, are mismatched at both class I and class II.

Detailed Description Text (132):
The methods of the invention are particularly useful for replacing a tissue or organ afflicted with a neoplastic disorder, particularly a disorder which is resistant to normal modes of therapy, e.g., chemotherapy or radiation therapy. Methods of the invention can be used for inducing tolerance to a graft, e.g., an allograft, e.g., an allograft from a donor which is mismatched at one or more class I loci, at one or more class II loci, or at one or more loci at each of class I and class II. In preferred embodiments: the graft includes tissue from the digestive tract or gut, e.g., tissue from the stomach, or bowel tissue, e.g., small intestine, large intestine, or colon; the graft replaces a portion of the recipient's digestive system e.g., all or part of any of the digestive tract or gut, e.g., the stomach, bowel, e.g., small intestine, large intestine, or colon.

Detailed Description Text (136):
As is discussed herein, hemoperfusion, e.g., hemoperfusion with a donor organ, can be used to deplete the host of natural antibodies. Other methods for depleting or otherwise inactivating natural antibodies can be used with any of the methods described herein. For example, drugs which deplete or inactivate natural antibodies, e.g., deoxyspergualin (DSG) Bristol), or anti-IgM antibodies, can be administered to the recipient of an allograft or a enograft. One or more of, DSG (or similar drugs),

anti-IgM antibodies, and emoperfusion, can be used to deplete or otherwise inactivate recipient natural antibodies in methods of the invention. DSG at a concentration of 6 mg/kg/day, i.v., has been found useful in suppressing natural antibody function in pig to cynomolgus kidney transplants.

Detailed Description Text (138):
Blockers of the CD40 ligand-CD40 or CD28-B7 interactions (or both) can be administered repeatedly. E.g., blockers can be administered one, two, three, or more times prior to donor bone marrow transplantation. Typically, a pre-bone marrow transplantation dose will be given to the patient at about 0 and -2 days. Additional, earlier doses 6, 7, or 8 days prior to bone marrow transplantation can also be given. It may be desirable to administer a first treatment then to repeat pre-bone marrow administrations every 1-5 days. Blockers can also be administered one, two, three, or more times after donor bone marrow transplantation. Typically, a post-bone marrow transplant treatment will be given about 2-14 days after bone marrow transplantation. The post bone marrow administration can be repeated as many times as needed. If more than one administration is given the administrations can be spaced about 1 week apart. Additional doses can be given if the patient appears to undergo early or unwanted T cell recovery. Preferably, blockers are administered at least once (and preferably two, three, or more times) prior to donor bone marrow transplantation and at least once (and preferably two, three, or more times) after donor bone marrow transplantation.

Detailed Description Text (140):
Some of the methods herein include the administration of hematopoietic stem cells to a recipient. In many of those methods, hematopoietic stem cells are administered prior to or at the time of the implantation of a graft (an allograft or a xenograft), the primary purpose of the administration of hematopoietic stem cells being the induction of tolerance to the graft. The inventors have found that one or more subsequent administrations (e.g., a second, third, fourth, fifth, or further subsequent administration) of hematopoietic stem cells can be desirable in the creation and/or maintenance of tolerance. Thus, the invention also includes methods in which hematopoietic stem cells are administered to a recipient, e.g., a primate, e.g., a human, which has previously been administered hematopoietic stem cells as part of any of the methods referred to herein.

Detailed Description Text (141):
While not wishing to be bound by theory the inventor believes that repeated stem cell administration may promote mixed chimerism and possibly long-term deletional tolerance in graft recipients. Accordingly, any method referred to herein which includes the administration of hematopoietic stem cells can further include multiple administrations of stem cells. In preferred embodiments: a first and a second administration of stem cells are provided prior to the implantation of a graft; a first administration of stem cells is provided prior to the implantation of a graft and a second administration of stem cells is provided at the time of implantation of the graft. In other preferred embodiments: a first administration of stem cells is provided prior to or at the time of implantation of a graft and a second administration of stem cells is provided subsequent to the implantation of a graft. The period between administrations of hematopoietic stem cells can be varied. In preferred embodiments a subsequent administration of hematopoietic stem cell is provided: at least two days, one week, one month, or six months after the previous administration of stem cells; at least two days, one week, one month, or six months after the implantation of the graft.

Detailed Description Text (142):
The method can further include the step of administering a second or subsequent dose of hematopoietic stem cells: when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the host donor specific antibody response, or by a change in the host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a

graft. Thus, method of the invention can be modified to include a further step of determining if a subject which has received a one or more administrations of hematopoietic stem cells is in need of a subsequent administration of hematopoietic stem cells, and if so, administering a subsequent dose of hematopoietic stem cells to the recipient.

Detailed Description Text (143):
Any of the methods referred to herein can include the administration of agents, e.g., 15-deoxyspergualin, mycophenolate mofetil, brequinar sodium, or similar agents, which inhibit the production, levels, or activity of antibodies in the recipient. One or more of these agents can be administered: prior to the implantation of donor tissue, e.g., one, two, or three days, or one, two, or three weeks before implantation of donor tissue; at the time of implantation of donor tissue; or after implantation of donor tissue, e.g., one, two, or three days, or one, two or three weeks after, implantation of a graft.

Detailed Description Text (144):
The administration of the agent can be initiated: when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the host donor specific antibody response, or by a change in the host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC's, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a graft.

Detailed Description Text (146):
Preferred embodiments include administration of 15-deoxyspergualin (6 mg/kg/day) for about two weeks beginning on the day of graft implantation.

Detailed Description Text (147):
Some of the methods referred to herein include the administration of hematopoietic stem cells to a recipient. The inventors have found that administration of one or more cytokines, preferably a cytokine from the species from which the stem cells are derived, can promote engraftment, mixed chimerism, and tolerance, or otherwise prolong acceptance of a graft. The use of such cytokines can reduce or eliminate the need for whole body irradiation. Thus, the invention also includes methods in the recipient is administered one or more cytokine, e.g., a donor-species cytokine.

Detailed Description Text (149):
Administration of a cytokine can begin prior to, at, or after the implantation of a graft or the implantation of stem cells.

Detailed Description Text (150):
The method can further include the step of administering a first or subsequent dose of a cytokine to the recipient: when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the host donor specific antibody response, or by a change in the host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC'S, e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a graft. Thus, method of the invention can be modified to include a further step of determining if a subject is in need of cytokine therapy and if so, administering a cytokine.

Other Reference Publication (1):
Lenschow, D.J. et al., (1995) "Inhibition of Transplant Rejection Following Treatment With Anti-B7-2 and Anti-B7-1 Antibodies", Transplantation, vol. 60, No. 10, pp 1171-1178.

Other Reference Publication (2):
Lenschow, D.J. et al., (1992) "Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA4lg", Science, vol. 257 (5071): 789-92.

Other Reference Publication (11):
Markees, T.G. et al.,(1997) "Prolonged survival of mouse skin allografts in recipients treated with donor splenocytes and antibody to CD40 ligand," Transplantation 64(2): 329-335.

Other Reference Publication (12):
Larsen, C.P., et al., (1996) "CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway," Transplantation 61(1): 4-9.

Other Reference Publication (13):
Elwood, E.T., et al., (1998) "Prolonged acceptance of concordant and discordant xenografts with combined CD40 and CD28 pathway blockade," Transplantation 65(11): 1422-1428.

Other Reference Publication (15):
Blazar, B.R., et al., (1994) "In vivo blockade of CD28/CTLA4: B7/BB1 interaction with CTLA4-lg reduces lethal murine graft-versus-host disease across the major histocompatibility complex barrier in mice," Blood 83(12): 3815-25.

Other Reference Publication (18):
Akalin, E., et al., (1996) "CD28-B7 T cell costimulatory blockade by CTLA4lg in the rat renal allograft model: inhibition of cell-mediated and humoral immune responses in vivo," Transplantation 62(12): 1942-5.

Other Reference Publication (20):
Sayegh, M.H., et al., (1997) "Donor antigen is necessary for the prevention of chronic rejection in CTLA4lg-treated murine cardiac allograft recipients," Transplantation 64(12): 1646-50.

Other Reference Publication (22):
Pearson, T.C., et al., (1997) "Analysis of the B7 costimulatory pathway in allograft rejection," Transplantation 63(10): 1463-9.

Other Reference Publication (23):
Pearson, T.C., et al., (1994) "Transplantation tolerance induced by CTLA4-lg [see comments]," Transplantation 57(12): 1701-6.

CLAIMS:

1. A method of promoting acceptance, by a recipient mammal, of a graft from a donor mammal of a second species comprising: administering to the recipient, an inhibitor of a costimulatory pathway; introducing into the recipient mammal, hematopoietic stem cells, and implanting the graft in the recipient.

7. The method of claim 2, wherein CTLA4-Ig and an anti-CD40L antibody are administered.

18. method of promoting acceptance, by a recipient mammal, of a graft from a donor mammal of the same species comprising: administering to the recipient, an inhibitor of a costimulatory pathway; introducing into the recipient mammal, hematopoietic stem cells; and preferably, implanting the graft in the recipient.

29. A method of promoting acceptance by a recipient mammal of a graft from a donor mammal comprising: administering to the recipient, an inhibitor of a costimulatory pathway; prior to or simultaneous with transplantation of the graft, introducing into the recipient mammal, donor thymic tissue; and implanting the graft in the recipient.

35. The method of claim 29, wherein CTLA4-Ig and an anti-CD40L antibody are administered.

46. A method of promoting acceptance, by a recipient mammal of a graft from a donor mammal of the same species, comprising: administering to the recipient, an inhibitor of a costimulatory pathway; introducing into the recipient mammal, hematopoietic stem cells, wherein the number of hematopoietic stem cells is sufficient such that mixed hematopoietic chimerism can be induced without whole body irradiation; and implanting the graft in the recipient.

52. The method of claim 46, wherein CTLA4-Ig and an anti-CD40L antibody are administered.

End of Result Set

Generate Collection

L9: Entry 59 of 59 File: DWPI Dec 21, 1995

DERWENT-ACC-NO: 1996-128992
DERWENT-WEEK: 200249

TITLE: Inhibition of antigen specific T cell responses - using an agent which inhibits a co-stimulatory signal in the T cell, opt. with other agents

Basic Abstract Text (1):
The following are claimed: (A) a method for inhibiting a response by a T cell to an antigen comprising administering (a) a first agent which inhibits a costimulatory signal in the T cell and (b) a second agent which (i) inhibits adhesion of the T cell to a cell which presents antigen to the T cell, or (ii) inhibits a proliferative signal in the T cell; (B) a method for inhibiting graft versus host disease (GVHD) in a bone marrow transplant recipient, comprising contacting a first population of cells comprising donor T cells in vitro with: (a) a second population of cells which express recipient alloantigens; (b) a first agent which inhibits a costimulatory signal in the donor T cells, and (c) a second agent which (i) inhibits adhesion of the donor T cells to cells which express recipient alloantigens, or (ii) inhibits a proliferative signal in the donor T cells, the first and second agents thereby inhibiting a response by the donor T cells to the cells which express recipient allo antigens such that, upon administration of the first population of cells to the bone marrow transplant recipient, GVHD in the recipient is inhibited; (C) a method for inhibiting GVHD in a donor bone marrow and donor T cell transplant recipient, comprising administering to the recipient: (a) a first agent which inhibits a costimulatory signal in the donor T cell and (b) a second agent which inhibits adhesion of the donor T cell to a cell presenting antigen to the T cell; (D) a method for inhibiting rejection of donor allogenic cells by a recipient comprising administering to the recipient: (a) a first agent which inhibits generation of a costimulatory signal in a recipient T cell and (b) a second agent which inhibits adhesion of a recipient T cell to a cell presenting antigen to the T cell; (E) a compsn. suitable for admin. comprising a human CTLA4-immunoglobulin fusion protein and an antihuman LFA-1 antibody or an anti-human interleukin-2 receptor (IL-2R) antibody and a carrier; (F) a compsn. suitable for admin. comprising: (a) a first agent selected from an anti-human By-1 monoclonal antibody (MAb) or fragment and/or an anti-human B7-2 MAb or fragment and (b) an anti-human LFA-1 antibody or anti-human IL-2R antibody and a carrier; (G) a method for inhibiting GVHD in a donor bone marrow and donor T cell transplant recipient, comprising administering to the recipient 1 agent which inhibits a costimulatory signal in the donor T cell.

Basic Abstract Text (2):
USE - The methods and compsns. can be used in vitro or in vivo to inhibit inappropriate T cell responses to antigens in clinical situations such as bone marrow and organ transplantation, allergic responses and autoimmune disorders, e.g. diabetes mellitus, arthritis, multiple sclerosis, dermatitis, psoriasis, asthma, leprosy reversal reactions, allergic encephalomyelitis, aplastic anaemia, chronic active hepatitis and prim. biliary cirrhosis.

End of Result Set

Generate Collection

L9: Entry 59 of 59 File: DWPI Dec 21, 1995

DERWENT-ACC-NO: 1996-128992
DERWENT-WEEK: 200249

TITLE: Inhibition of antigen specific T cell responses - using an agent which inhibits a co-stimulatory signal in the T cell, opt. with other agents

Basic Abstract Text (1):
The following are claimed: (A) a method for inhibiting a response by a T cell to an antigen comprising administering (a) a first agent which inhibits a costimulatory signal in the T cell and (b) a second agent which (i) inhibits adhesion of the T cell to a cell which presents antigen to the T cell, or (ii) inhibits a proliferative signal in the T cell; (B) a method for inhibiting graft versus host disease (GVHD) in a bone marrow transplant recipient, comprising contacting a first population of cells comprising donor T cells in vitro with: (a) a second population of cells which express recipient alloantigens; (b) a first agent which inhibits a costimulatory signal in the donor T cells, and (c) a second agent which (i) inhibits adhesion of the donor T cells to cells which express recipient alloantigens, or (ii) inhibits a proliferative signal in the donor T cells, the first and second agents thereby inhibiting a response by the donor T cells to the cells which express recipient allo antigens such that, upon administration of the first population of cells to the bone marrow transplant recipient, GVHD in the recipient is inhibited; (C) a method for inhibiting GVHD in a donor bone marrow and donor T cell transplant recipient, comprising administering to the recipient: (a) a first agent which inhibits a costimulatory signal in the donor T cell and (b) a second agent which inhibits adhesion of the donor T cell to a cell presenting antigen to the T cell; (D) a method for inhibiting rejection of donor allogenic cells by a recipient comprising administering to the recipient: (a) a first agent which inhibits generation of a costimulatory signal in a recipient T cell and (b) a second agent which inhibits adhesion of a recipient T cell to a cell presenting antigen to the T cell; (E) a compsn. suitable for admin. comprising a human CTLA4-immunoglobulin fusion protein and an antihuman LFA-1 antibody or an anti-human interleukin-2 receptor (IL-2R) antibody and a carrier; (F) a compsn. suitable for admin. comprising: (a) a first agent selected from an anti-human By-1 monoclonal antibody (MAb) or fragment and/or an anti-human B7-2 MAb or fragment and (b) an anti-human LFA-1 antibody or anti-human IL-2R antibody and a carrier; (G) a method for inhibiting GVHD in a donor bone marrow and donor T cell transplant recipient, comprising administering to the recipient 1 agent which inhibits a costimulatory signal in the donor T cell.

Basic Abstract Text (2):
USE - The methods and compsns. can be used in vitro or in vivo to inhibit inappropriate T cell responses to antigens in clinical situations such as bone marrow and organ transplantation, allergic responses and autoimmune disorders, e.g. diabetes mellitus, arthritis, multiple sclerosis, dermatitis, psoriasis, asthma, leprosy reversal reactions, allergic encephalomyelitis, aplastic anaemia, chronic active hepatitis and prim. biliary cirrhosis.

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File 410:Chronolog(R)  1981-2003/Dec
       (c) 2003 The Dialog Corporation

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*File   5: BIOSIS Previews has been reloaded with major enhancements.
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 4/3/1     (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
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  126017804    CA: 126(2)17804h    PATENT
  Human antibodies derived from immunized xenomice
  INVENTOR(AUTHOR): Kucherlapati, Raju; Jakobovits, Aya; Klapholz, Sue;
Brenner, Daniel G.; Capon, Daniel J.
  LOCATION: USA
  ASSIGNEE: Cell Genesys, Inc.
  PATENT: PCT International ; WO 9634096 A1  DATE: 19961031
  APPLICATION: WO 95US5500 (19950428)
  PAGES: 64 pp.  CODEN: PIXXD2  LANGUAGE: English  CLASS: C12N-015/00A
  DESIGNATED COUNTRIES: AU; CA; FI; HU; JP; KR; NO; NZ
  DESIGNATED REGIONAL: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LU; MC;
NL; PT; SE


 4/3/2     (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
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124173443 CA: 124(13)173443d PATENT
Methods for inhibiting antigen specific T cell responses
INVENTOR(AUTHOR): Blazar, Bruce R.; Vallera, Daniel A.
LOCATION: USA
ASSIGNEE: Regents of the University of Minnesota
PATENT: PCT International ; WO 9534320 A2 DATE: 951221
APPLICATION: WO 95US7351 (950607) *US 255267 (940607) *US 472697 (950606)
PAGES: 61 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-039/00A; C07K-014/705B; C07K-014/725B; C07K-016/28B; C07K-019/00B
DESIGNATED COUNTRIES: AU; CA; JP DESIGNATED REGIONAL: AT; BE; CH; DE; DK ; ES; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE
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L4: Entry 1 of 11 File: PGPB Jul 3, 2003

DOCUMENT-IDENTIFIER: US 20030124614 A1
TITLE: Novel T-cell membrane protein (TIRC7), peptides and antibodies derived therefrom and uses thereof

Abstract Paragraph (1):
Described are generally a T cell immune response cDNA 7 (TIRC7) encoding a novel T-cell transmembrane protein as well as peptides und polypeptides derived therefrom and antibodies recognizing said (poly)peptides. More particularly, peptide and polypeptide as well as antibodies being capable of inhibiting T-cell stimulation through the T-cell membrane protein (TIRC7) are provided. Furthermore, vectors comprising the aforementioned polynucleotides and host cells transformed therewith as well as their use in the production of the above-defined proteins, peptides or polypeptides are described. Additionally, pharmaceutical and diagnostic compositions are provided comprising any one of the afore described polynucleotide, vector, protein, peptide, polypeptide, or antibody. Furthermore, methods and uses for modulating immune responses through the novel TIRC7 membrane protein as well as pharmaceutical compositions comprising agents which act on the TIRC7 membrane protein or its ligand are described. Also, the use of said polynucleotide, vector, protein, peptide, polypeptide, or antibody for the preparation of pharmaceutical compositions for use in organ transplantation, for the treatment of autoimmune, allergic or infectious diseases, or for treatment of tumors is provided.

Summary of Invention Paragraph (2):
[0001] The present invention pertains generally to a T cell immune response cDNA 7 (TIRC7) encoding a novel T-cell transmembrane protein as well as peptides und polypeptides derived therefrom and antibodies recognizing said (poly)peptides. In a first aspect, the present invention relates to TIRC7 cDNA and its encoded protein. In a further aspect, the present invention relates to polynucleotides derived from said TIRC7 cDNA encoding a peptide or polypeptide being capable of inhibiting T-cell stimulation through the T-cell membrane protein (TIRC7). Furthermore, the present invention relates to vectors comprising such polynucleotides and host cells transformed therewith as well as their use in the production of the above-defined peptides or polypeptides. In addition, the present invention relates to the (poly)peptide encoded by said polynucleotides or obtainable by the method of the invention. In another important aspect the present invention relates to antibodies against said peptides and polypeptides that are capable of inhibiting T-cell stimulation through the T-cell membrane protein (TIRC7). The present invention additionally relates to pharmaceutical and diagnostic compositions comprising the aformentioned peptide, polypeptide, or antibody. Furthermore, the present invention relates to methods and uses for modulating immune responses through the novel TIRC7 membrane protein as well as to pharmaceutical compositions comprising agents which act on the TIRC7 membrane protein or its ligand. Also, the invention relates to the use of the before-described polynucleotide, vector, peptide, polypeptide, or antibody for the preparation of pharmaceutical compositions for use in organ transplantation, for the treatment of autoimmune, allergic or infectious diseases, or for treatment of tumors. Furthermore, the present invention relates to methods for modulating (antigen-specific) T cell unresponsiveness. The present invention encompasses methods for inducing, maintaining or reversing T cell unresponsiveness by inhibiting or stimulating an (unresponsive) T cell through the novel TIRC7 membrane protein.

Summary of Invention Paragraph (5):
[0003] T cell activation is a serial process involving multiple signaling pathways and sequential changes in gene expression resulting in differentiation of T cells into

distinct subpopulations, i.e. Th1 and Th2, which are distinguishable by their pattern of cytokine production and characterize the mode of the cellular immune response (Abbas et al., 1996; Crabtree, 1989). The T cell response is initiated by the interaction of the antigen-specific T cell receptor (TCR) with peptide presented by major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells (APCs). Additional signals are provided by a network of receptor-ligand interactions mediated by a number of membrane proteins such as CD28/CTLA4 and B7, CD40/CD40L, LFA-1 and ICAM-1 (Lenschow et al., 1996; Linsley and Ledbetter, 1993; Xu et al., 1994, Bachmann et al., 1997; Schwartz, 1992), collectively called costimulatory signals (Perez et al., 1997). These membrane proteins can alter T cell activation in distinct ways (Bachmann et al., 1997) and regulate the immune response by the integration of positive and negative signals provided by these molecules (Bluestone, 1995; Perez et al., 1997). Many of the agents which are effective in modulating the cellular immune response either interfere with the T cell receptor (Cosimi et al., 1981), block costimulatory signaling (Larsen et al., 1996; Blazar et al., 1996; Kirk et al., 1997; Linsley et al., 1992; Turka et al., 1992) or inhibit intracellular activation signals downstream from these primary cell membrane triggers (Schreiber and Crabtree, 1992). Therapeutic prevention of T cell activation in organ transplantation and autoimmune diseases presently relies on panimmunosuppressive drugs interfering with downstream intracellullar events. Specific modulation of the T cell response remains a longstanding goal in immunological research.

Summary of Invention Paragraph (7):
[0004] The present invention relates to polynucleotides encoding a novel T-cell membrane protein. Furthermore, the present invention relates to peptides and polypeptides derived therefrom as well as to antibodies capable of inhibiting T-cell stimulation through the novel T-cell membrane protein. More particulary, the present invention relates to applications in the medical field that directly arise from the polynucleotides, peptides, (poly)peptides and antibodies of the invention. Additionally, the present invention relates to a novel method for testing activators and inhibitors of T-cell proliferation. The pharmaceutical compositions, methods and uses of the invention are useful therapeutically in situations where it is desirable to modulate (antigen-specific) immune responses, e.g., inducing and maintain (antigen-specific) T-cell unresponsiveness or restore (antigen-specific) T-cell responsiveness. For example, it may be necessary to induce or maintain T-cell unresponsiveness in a subject who has received an organ or bone marrow transplant to prevent graft rejection by inhibiting stimulation through the TIRC7 membrane protein. In addition, T-cell unresponsiveness can be maintained by blocking TIRC7 stimulation in a subject who has an autoimmune disease to alleviate symptoms of the autoimmune disease. In these cases, a TIRC7 inhibitory agent is administered to the subject in an amount and over a period of time sufficient to maintain T-cell unresponsiveness. Alternatively, T-cell unresponsiveness can be reversed in a subject bearing a tumor to stimulate a tumor specific T-cell response or in a subject receiving a vaccine to enhance the efficacy of the vaccine. For example, a cell (e.g., a tumor cell) can be modified to express a TIRC7 ligand or a TIRC7 stimulatory agent can be administered to the subject bearing a tumor or who has had a tumor surgically removed to prevent recurrence of the tumor. Additionally, antigen-specific responsiveness can be restored to anergized T-cells in vitro by stimulating the T-cells through TIRC7. Responsive T-cells generated in vitro can then be administered to a subject.

Summary of Invention Paragraph (9):
[0005] In view of the need of therapeutic means for the treatment of diseases related to immune responses of the human body, the technical problem of the invention is to provide means and methods for the modulation of T-cell responses which are particularly useful in organ transplantation and autoimmune diseases.

Summary of Invention Paragraph (52):
[0048] While the above described results hold promise that the novel TIRC7 protein, and antibodies thereto may be therapeutically useful, proof for the concept of the invention, namely the usefulness of the above described-compounds for the modulation of the immune response as well as the embodiments derived therefrom and characterized hereinbelow came from further experiments performed in accordance with the present invention demonstrating the ability of an anti-TIRC7 antibody to prevent allograft rejection in the in vivo model of rat kidney transplantation; see Example 4, FIGS. 6 und 7. Moreover, it could be demonstrated in accordance with the present invention

that advantageously the effects of antibody targeting of TIRC7 are quite similar to those observed by targeting of costimulatory molecules. Antibody blocking of costimulatory molecules has been shown to inhibit human T cell proliferation (Linsley et al., 1992; Walunas et al., 1994). Furthermore, interruption of CD28/B7 interaction with the soluble protein CTLA4Ig caused inhibition of T cell proliferation (Linsley et al., 1992; Lenschow et al., 1992; Larsen et al., 1996). Further analogy to the effect of TIRC7 antibody targeting is provided by CTLA4Ig selectively blocking Th1 and sparing Th2 lymphocyte responses (Mohammed et al., 1995). It was shown, that administration of CTLA4Ig in an in vivo model of kidney allograft transplantation prolonged graft survival (Mohammed et al., 1995) which was similarly observed by TIRC7 antibody targeting in the present Examples. Although these similarities may suggest a costimulatory function, TIRC7 does not share structural or sequence homology with any of the known T cell accessory molecules. Thus, TIRC7 may participitate in a distinct signaling pathway induced early in the course of T cell activation. This possibility is supported by recent reports that interference with pathways mediated by molecules other than the known costimulatory proteins can modulate the T cell response. For example, antibody targeting of the common leukocyte antigen CD45RB was shown to result in a prevention of graft rejection in mice (Lazarovits et al., 1996). Given the functional similarities between TIRC7 and the known T cell accessory molecules, it is expected that the structural novelty of TIRC7 will contribute to the understanding of distinct mechanisms in the T cell response. Moreover, the striking capacity of anti-TIRC7 antibody to significantly prolong allograft survival in vivo provide a novel approach for a selective inhibition of undesired T cell activation in human organ transplantation and autoimmune diseases.

Summary of Invention Paragraph (83):
[0079] Such agents comprise those blocking the activity of, e.g., costimulatory molecules, integrins, Ig-superfamily molecules, selectins as well as drugs blocking chemokines and their respective receptor interactions, drugs blocking IL2/IL2-receptor interaction and other conventional immunosuppressive drugs such as TL-2R mAbs, IL-Toxins and IL-Muteins. Examples for costimulatory molecules and their ligands are described in the prior art, e.g., in Schwartz, Cell 71 (1992), 1065-1068. The interruption of the receptor/ligand interactions by using mAbs or soluble CTLA4Ig for the interaction between CD28 to the B7-2 and CTLA4 to B7-1 and B7-2 are described in Blazar, J. Immunol. 157 (1996), 3250-3259; Bluestone, Immunity 2 (1995), 555-559; Linsley, Science 257 (1992), 792-95. Examples for blocking the receptor/ligand interaction by using mAbs to CD40 or CD40L are reported by Burden, Nature 381 (1996), 434-435; Kirk, Proc. Natl. Acad. Sci. USA 94 (1997), 8789-8794. CD2 antigen and its ligand LFA-3 are described in Bagogui Li et al., review in Adhesion Molecules, Fusion proteins, Novel Peptides, and Monoclonal Antibodies, Recent Developments in Transplantation Medicine, Vol. II, 1995, Physicians&Scientists Publishing Co., Inc. and blocking of their interaction by using of mAbs (anti-Leu-5b, OKT11, T11) is reported in Brumberg, Transplantation 51 (1991) 219-225 or CD2.lgG1 fusion protein. The use of monoclonal Abs agains CD4 molecule is described in Cosimi, Surgery 108 (1990), 406-414. CD47 blockade by mAbs is described by Rheinhold, J. Exp. Med. 185 (1997), 1-11. Integrins and lg-superfamily molecules include LFA-1 with its ligand ICAM-1, -2, -3, Mac-1 with ist ligand ICAM-1, -3; ICAM-1 with its ligand LFA-1, Mac-1, CD43; ICAM-2 with ist ligand LFA-1; ICAM-3 with its ligand LFA-1, Mac-1; VLA4 and VCAM-1 see, e.g., David, Adams, review in Adhesion Molecules, Fusion proteins, Novel Peptides, and Monoclonal Antibodies, Recent Developments in Transplantation Medicine, Vol. II, 1995, Physicians&Scientists Publishing Co., Inc.; Isobe, Science, 255 (1992), 1125-1127; Cosimi, J. Immunology 144 (1990), 4604-4612; Hynes, Cell 69 (1992),11-25.

Summary of Invention Paragraph (85):
[0081] Another example is the drug pentoxifylline (PTX) that is able to block expression of VCAM-1; Besler, J. Leukoc. Biol. 40 (1986), 747-754. Furthermore, VCAM-1 mAb, M/K-2, anti-murine, for example could prolong allograft survival, Orosz, Transplantation, 56 (1993), 453-460.

Summary of Invention Paragraph (86):
[0082] Blocking of members of the integrin family and their ligands by using mAbs is decribed in Kupiec-Weglinski, review in Adhesion Molecules, Fusion proteins, Novel Peptides, and Monoclonal Antibodies, Recent Developments in Transplantation Medicine, Vol. II, 1995, Physicians&Scientists Publishing Co., Inc.

Summary of Invention Paragraph (88):
[0084] The combination of conventional immunosuppressive drugs, e.g., ATG, ALG, OKT3, Azathioprine, Mycophenylate, Mofetyl, Cyclosporin A, FK506, Corticosteroids may be used as decribed in Cosimi, Transplantation 32 (1981), 535-539; Shield, Transplantation 38 (1984), 695-701.

Summary of Invention Paragraph (91):
[0087] Advantageously, the pharmaceutical composition of the invention is intended for use in organ transplantation, for the treatment of autoimmune, allergic or infectious diseases, or for the treatment of tumors. An example for the use of the pharmaceutical composition of the invention for improving allograft or xenograft tolerance is described with respect to administration of an LFA-3 and CD2 binding protein, respectively, in WO93/06852.

Summary of Invention Paragraph (106):
[0102] In a particularly preferred embodiment of the invention, the uses, methods and pharmaceutical compositions are intended to be applied to a subject who is a recipient of bone marrow transplant or peripheral stem cell transplant. Preferably the pharmaceutical composition is designed for contacting with bone marrow or peripheral stem cell prior to transplantation into the recipient.

Summary of Invention Paragraph (107):
[0103] In a further particular preferred embodiment, the methods and uses of the present invention are applied in organ graft transplantation, peripheral stem cell transplantation or for the treatment of auto-immune diseases or allergy.

Summary of Invention Paragraph (116):
[0112] Compounds found to activate T-cell mediated responses may be used in the treatment of cancer and related diseases. In addition, it may also be possible to specifically inhibit viral diseases, thereby preventing viral infection or viral spread. Compounds identified as suppressors of T-cell activation or stimulation can be used, e.g., for treating skin conditions (see, e.g., WO93/06866) or in allogenic or xenogenic cell or organ transplantation in order to avoid graft rejection; see also supra.

Summary of Invention Paragraph (122):
[0118] Furthermore, the present invention relates to the use of the polynucleotide, the vectors, peptides, polypeptides, antibodies and cells of the invention as well as compounds identified in accordance with a method of the invention described hereinabove for the preparation of a pharmaceutical composition for the treatment of diseases involving T-cell activation and associated with Th1 and Th2 immune response, for the treatment of acute and chronic rejection of allo- and xeno organ transplants and bone marrow transplantation, for the treatment of rheumatoid arthritis, lupus erythramatodes, multiple sklerosis, encephalitis, vasculitis, diabetes mellitus, pancreatitis, gastritis, thyroiditis, for the treatment of maligne disorders of T, B or NK cells, for the treatment of asthma, lepramatosis, Helicobacter pylori associated gastritis or for the treatment of skin tumors, adrenal tumors or lung tumors.

Brief Description of Drawings Paragraph (8):
[0127] FIG. 6: Anti-TIRC7 antibody targeting in vivo significantly prolongs allograft survival. (A). Lewis rat recipients of Wistar Furth rat kidney allografts received either anti-TIRC7 Ab73 (n=7), control antibody from preimmune serum (n=7), or no treatment (n=7). Treatment was initiated at 2 h prior to and immediately after transplantation, and was repeated on day 1, 2, 4, and 6 post-transplantation. Animals treated with control antibody showed a mean survival time of 8.+-.1 days whereas mean survival time of animals representing the untreated control group was 7.+-.2 days. Six of the seven animals in the experimental group maintained functional grafts for more than 45 days. One rat in the anti-TIRC7 antibody treated experimental group had a survival time of 21 days. As assessed at day 45 after transplantation, the mean survival time in this group was 41,5 days ($p<0.001$ vs controls).

Brief Description of Drawings Paragraph (9):
[0128] FIG. 7: Histological analysis of kidney allografts at day 7 post-transplantation. (A). Kidney allografts of rats receiving control antibodies showed severe tissue destruction and diffuse mononuclear infiltration which was

similar to histological findings in the kidney allografts of untreated animals. (B). Renal allografts of two additional anti-TIRC7 antibody treated animals sacrificed at day 7 showed very mild interstitial infiltration of mononuclear cells. Tissue lessions were not identified in the allografts of these animals.

Detail Description Paragraph (19):
[0142] The effect of modulating the TIRC7 mediated signal was studied in an animal model featuring kidney transplantation from Wistar Furth to Lewis rats. Male inbred rats 200-250 g (Harlan Winkelmann, Germany) were used throughout the experiment. Wistar Furth rats (WF, RT1.sup.u) were grafted into bilaterally nephrectomized Lewis rats (LEW, RT1.sup.1) using microsurgical techniques; ischemic time was 30.+-.5 min. Cryostat sections were fixed in formalin. The fixed tissue was paraffin embedded, and tissue sections were stained with hematoxylin and eosin. In initial experiments, anti-human TIRC7 antibodies were tested for their ability to inhibit the proliferation of

Detail Description Paragraph (20):
[0143] Lewis rat lymphocytes stimulated with irradiated Wistar Furth rat lymphocytes in vitro. Ab73 was shown to profoundly block rat T cell proliferation. In kidney transplant experiments, animals remained either untreated (n=7), received preimmune rabbit serum (n=7) or were treated with anti-TIRC7 antibody Ab73 (n=7), 2 h before, directly after and on days 1, 2, 4 and 6 after transplantation. No side effects except for transient mild diarrhea were observed in the anti-TIRC7 antibody treated group. Anti-TIRC7 antibody significantly prolonged the graft survival time of treated animals (p<0, 001). Six of seven allografts of the anti-TIRC7 treated animals remained functional for more than 45 days after completion of antibody administration. One animal treated with anti-TIRC7 antibody died at day 21. In contrast, all animals in both control groups died of renal failure by day 7 to 9 after transplantation (FIG. 6). Histological examination of kidney grafts from two additional antibody treated animals sacrificed at day 7 post-transplantation demonstrated very mild lymphocytic infiltration but no signs of tissue necrosis (FIG. 7B). In contrast, kidney grafts from control animals displayed remarkable evidence of acute graft rejection including diffuse mononuclear cell infiltrates as well as extensive areas of necrosis (FIG. 7A).

Detail Description Paragraph (25):
[0148] Antibody targeting of TIRC7 has a selective inhibitory effect on the Th1 lymphocyte subset, as evidenced by the inhibition of IL-2 and IFN-g, but not IL-4, cytokine production. With anti-TIRC7 antibody treatment the cells appear to remain in an unresponsive, but functional, state since exogenous recombinant IL-2 reversed the antiproliferative effect of the anti-TIRC7 antibodies. The ability of an anti-TIRC7 antibody to prevent allograft rejection in the in vivo model of rat kidney transplantation reflects the findings obtained in the in vitro studies. Moreover, the effects of antibody targeting of TIRC7 are quite similar to those observed by targeting of costimulatory molecules. TIRC7 does not share structural or sequence homology with any of the known T cell accessory molecules. Thus, TIRC7 may participitate in a distinct signaling pathway induced early in the course of T cell activation.

Detail Description Paragraph (26):
[0149] Given the functional similarities between TIRC7 and the known T cell accessory molecules, it is expected that the structural novelty of TIRC7 will contribute to the understanding of distinct mechanisms in the T cell response. Moreover, the striking capacity of anti-TIRC7 antibody to significantly prolong allograft survival in vivo provide a novel approach for a selective inhibition of undesired T cell activation in human organ transplantation and autoimmune diseases.

Detail Description Paragraph (34):
[0157] Cosimi, Transplantation 32 (1981), 535-539

CLAIMS:

16. The pharmaceutical composition of any one of claims 10 to 15 for use in cell or organ transplantation, for the treatment of autoimmune, allergic or infectious diseases, or for the treatment of tumors.

37. The use of any one of claims 19 to 22 or 33 to 35, wherein the subject is a recipient of peripheral stem cells or bone marrow transplant.

38. The use of claim 37, wherein the pharmaceutical composition is designed for contacting with peripheral stem cells or bone marrow cell prior to transplantation into the recipient.

39. The method of any one of claims 18, 20 to 22 or any one of claims 27 to 32 or the use of any one of claims 19 to 22 or 33 to 36 in organ graft transplantation or for the treatment of auto-immune diseases.

42. Use of peptide or polypeptide being capable of inhibiting T-cell stimulation through the TIRC7 membrane protein and/or being recognized by an antibody capable of inhibiting T-cell stimulation through the TIRC7 membrane protein encoded by a fragment of the polynucleotide of claim 1 or an antibody specifically recognizing said peptide or polypeptide the polynucleotide of claim 1, the vector of claim 3 or 4, the protein of claim 7, the antibody of claim 8, the cell of claim 5 or 9 or the compound indentified according to the method of claim 40 for the preparation of a pharmaceutical composition for the treatment of acute and chronic diseases, involving T-cell activation and Th1 and Th2 immune response, for the treatment of acute and chronic rejection of allo- and xeno organ transplants and bone marrow transplantation, for the treatment of rheumatoid arthritis, lupus erythramatodes, multiple sklerosis, encephalitis, vasculitis, diabetes mellitus, pancreatitis, gastritis, thyroiditis, for the treatment of maligne disorders of T, B or NK cells, for the treatment of asthma, lepramatosis, Helicobacter pylori associated gastritis or for the treatment of skin tumors, adrenal tumors or lung tumors.

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L4: Entry 4 of 11 File: PGPB Jan 2, 2003

DOCUMENT-IDENTIFIER: US 20030003098 A1
TITLE: Inhibiting rejection of a graft

Abstract Paragraph (1):
Disclosed are methods for inhibit rejection of a graft in a patient. The methods involve treating the graft with a molecule which binds to a co-stimulatory protein of antigen-presenting cells. Useful molecules include chimeras having enzymatically inactive polypeptides bonded to polypeptides which bind to co-stimulatory proteins of antigen-presenting cells. Also disclosed, are chimeric molecules composed of lytic IgG Fc bonded to CD2, CD28, CD40L, or CTLA-4. In addition, disclosed are methods for inhibiting rejection of a graft in a patient; the methods involve treating the brain-dead, beating heart donor of the graft, prior to removal of the graft from the donor, to render the graft less susceptible to rejection by the patient.

Summary of Invention Paragraph (2):
[0001] This invention relates to inhibiting rejection of a graft in a patient.

Summary of Invention Paragraph (3):
[0002] T-cells play an important role in the rejection of allografts and xenografts (also referred to herein as "grafts"). Activation of T-cells bearing clonotypic receptors for donor alloantigen requires two distinct signals. The binding of a T-cell receptor to an alloantigen serves as one signal. The second signal, which is not delivered via the T-cell receptor, has been termed a co-stimulatory signal. The co-stimulatory signal is based on the interaction of ligands on the surfaces of antigen presenting cells (APCs) and T-cells (for a review, see Janeway et al., 1994, Cell 76: 275). For example, members of the B7 family of co-stimulatory proteins, including B7-1, B7-2, and B7-3, are expressed on APCs and interact with the CD28 T-cell surface protein. Engagement of the CD2 protein on T-cells with LFA-3 or CD48 on APCs also provides a co-stimulatory signal. After receiving both signal one and signal two, a T-cell proliferates and differentiates into an armed effector cell. T-cells that bind antigen without receiving a co-stimulatory signal are thought to undergo apoptosis or to become anergic (i.e., they fail to proliferate in response to antigenic rechallenge).

Summary of Invention Paragraph (4):
[0003] In a mixed lymphocyte culture (MLC), the T-cell proliferative response to alloantigen can be inhibited by blocking binding of B7 to CD28 (Tan et al., 1993, J. Exp. Med. 177: 165-173). In such an in vitro system, binding can be blocked in the presence of CTLA-4Ig, a chimeric immunoglobulin fusion protein incorporating the extracellular domain of CTLA-4. The extracellular domains of CTLA-4 and CD28 have considerable homology. CTLA-4 or CTLA-4Ig, however, binds B7 with higher affinity than does CD28. The systemic application of CTLA-4Ig promotes engraftment and can lead to tolerance of the graft when administered to recipient mice upon transplantation of pancreatic islet cells (Lenschow et al., Science, 1992, 257: 789).

Summary of Invention Paragraph (6):
[0004] I have found that rejection of a graft containing a cell which expresses a co-stimulatory protein(s) can be inhibited by treating (i.e., coating) the graft, in lieu of treating the recipient of the graft (i.e., the patient), to inhibit generation of a co-stimulatory signal and activation of host T-cells by the graft. Accordingly, in one aspect, the invention features inhibiting rejection of a graft containing a cell which expresses a co-stimulatory protein in a patient (e.g., a human) involving treatment of the graft in the patient with a molecule, other than lytic CTLA-4/Fc,

which binds to a co-stimulatory protein that is expressed upon antigen-presenting cells, thereby inhibiting activation of host T-cells by the graft. In embodiments of this aspect of the invention, the graft can also be treated ex vivo and/or in the donor (e.g., a brain-dead, beating-heart donor).

Summary of Invention Paragraph (7):
[0005] In a second aspect, the invention features a method for inhibiting rejection of a graft containing a cell that expresses a co-stimulatory protein in a patient, involving treating the graft outside of the patient with a molecule which binds to a co-stimulatory protein of antigen presenting cells, thereby inhibiting activation of host T-cells by the graft. The graft is treated ex vivo (i.e., in vitro) or, preferably, the graft is treated in a brain-dead, beating heart donor. If desired, the graft can be treated with a combination of methods. For example, the graft can be treated (1) in the brain-dead, beating-heart donor and ex vivo, (2) ex vivo and in the patient (e.g., by perfusing a chimeric molecule into the graft, with closure of the surgical anastomosis between the donor and the patient), (3) in the brain-dead, beating-heart donor and in the patient, or (4) in the brain-dead, beating-heart donor, ex vivo, and in the patient.

Summary of Invention Paragraph (8):
[0006] Suitable molecules for use in the first and second aspects of the invention include CTLA-4, CD28, CD40L (i.e., CD40 ligand), and/or CD2. Other suitable molecules include chimeric molecules that have (i) a first polypeptide which binds to a co-stimulatory protein of antigen-presenting cells bonded to (ii) a second polypeptide, the second polypeptide being one which is enzymatically inactive (e.g., non-lytic IgG heavy chains or portions thereof) in humans and which increases the circulating half-life of the first polypeptide by a factor of at least two. Where the graft is treated outside of the patient, monoclonal antibodies which specifically bind to co-stimulatory proteins of antigen-presenting cells can be used to treat the graft. These monoclonal antibodies can be identified by their ability to block the ectodomain of T-cell surface proteins from binding to co-stimulatory proteins on antigen-presenting cells. Suitable monoclonal antibodies include those which specifically bind to CD48, CD40, LFA-3, or a B7 protein such as B7-1, B7-2, or B7-3 (see, e.g., Gimmi et al., 1991, Proc. Nat'l. Acad. Sci. 88:6575-6579; Freeman et al., 1989, J. Immunol. 143:2714-2722; Boussiotis et al., 1993, Proc. Nat'l. Acad. Sci. 90:11059-11063; and Engel et al., 1994, Blood 84: 1402-1407).

Summary of Invention Paragraph (12):
[0010] If desired, the graft can be treated with a combination of molecules. For example, the graft can be treated with CD28 or CTLA-4 ex vivo and then with a lytic CD2/Fc chimera in the patient. In preferred combinations, the graft is treated with a CTLA-4/Fc chimera and with a CD2/Fc chimera, either simultaneously or sequentially.

Summary of Invention Paragraph (13):
[0011] In another aspect, the invention features chimeric molecules having a first polypeptide which includes CD2, CTLA-4, CD28, or CD40L covalently bonded to a second polypeptide which includes non-lytic IgG Fc. Preferred molecules include IgG Fc covalently bonded to a hinge region which is covalently bonded to CD2, CTLA-4, CD40L, or CD28. The aforementioned molecules are useful in inhibiting rejection of a graft in the methods described herein.

Summary of Invention Paragraph (14):
[0012] The invention also features inhibiting rejection of a graft in a patient, involving treating the brain-dead, beating-heart donor of the graft, prior to removal of the graft from the donor, to render the graft less susceptible to posttransplantation rejection by the patient. In a preferred embodiment, treatment involves modifying, eliminating, or masking a cell-surface protein of the graft. The cell-surface protein can be one which is capable of causing a co-stimulatory signal in T-lymphocytes in the patient (e.g., a co-stimulatory protein such as a B7 protein), or the cell-surface protein can be any antigen which is capable of causing a T-lymphocyte-mediated response in the patient (e.g., ICAM-1). The cell-surface antigen or co-stimulatory protein can be masked by treating the graft with a non-lytic masking agent which includes an antibody F(ab').sub.2 fragment which is capable of forming a complex with an antigen or co-stimulatory protein on the cell. If desired, a cell bearing a co-stimulatory protein can be lysed with a chimeric molecule which has (i) a

- polypeptide which binds to a co-stimulatory protein fused to (ii) a polypeptide which has a lytic Fc region of an IgG molecule and which lacks an IgG heavy chain variable region.

Summary of Invention Paragraph (15):
[0013] By "graft" is meant any cell, tissue, or organ (e.g., islet cells, and kidney, heart, liver, lung, brain, and muscle tissues) transplanted from one individual (e.g., a mammal such as a human) to another.

Summary of Invention Paragraph (21):
[0019] The invention provides a method for inhibiting rejection of a graft; accordingly, the invention is useful for protecting the graft from rejection and promoting tolerance of a transplanted cell, organ, or tissue. One advantage of the invention is that it obviates systemic immunosuppression of the patient. Treating the graft outside of the patient blocks co-stimulation by donor graft antigens and leaves normal protective immune responses to non-graft antigens unimpaired.

Brief Description of Drawings Paragraph (8):
[0028] FIG. 7 is a graph indicating that islet cell allograft treatment with (NL) CTLA-4/Fc prolongs engraftment. Fresh islet cell isolates harvested from DBA/2J mice were incubated for 1 hour prior to implantation with either media alone, 10 .mu.g/ml mIgG3 (control protein), or 10 .mu.g/ml (NL) mCTLA-4/Fc. Subsequently, 300-400 islets were injected under the left renal capsule of streptozotocin-treated diabetic B6AF1 recipients, and graft function was followed by monitoring blood glucose levels.

Brief Description of Drawings Paragraph (9):
[0029] FIGS. 8A-D are a series of photographs obtained during a histologic analysis of islet grafts in tolerant hosts. FIG. 8A is a photograph indicating that tolerance to an islet allograft treated with (NL) CTLA-4/Fc is not synonymous with the absence of an allograft response (hematoxylin and eosin staining; 200X); M, mononuclear cell infiltrate; S, intact islet. FIG. 8B is a photograph showing cells stained with rat anti-mouse CD4 monoclonal Ab (200X), and FIG. 8C is a photograph indicating that cells stained with rat anti-mouse CD8.sup.+ monoclonal Ab (200X) surround, but do not invade, the islet allografts in tolerant mCTLA-4/Fc treated hosts. FIG. 8D is a photograph displaying the results of immunohistology of a graft incubated with the exclusion of a primary antibody (200X).

Detail Description Paragraph (11):
[0040] Treating the Graft: Ex vivo treatment of the graft can be accomplished with standard techniques (including the use of infusion pumps and syringes) for perfusing fluids into organs, cells, or tissues. If desired, conventional immunohistology methods can be used to assay the degree to which the graft is coated with the molecule which binds the co-stimulatory protein (see, e.g., Brewer et al., 1989, The Lancet 2:935). Generally, the concentration of the molecule will be 0.1 to 10 mg/ml; preferably, the concentration is 0.5 to 2 mg/ml. If desired, the graft can simply be immersed in a solution of the desired chimeric molecule(s) (e.g., CTLA-4/FC) and a physiologically acceptable carrier (e.g., saline). Generally, the graft will be incubated for 30 minutes to 1 week; preferably, where intact organs are used, the intact organ is incubated for approximately 30 minutes, and where cultured cells are used, cultured cells are incubated for several days. Generally, for immersion, the concentration of the molecule which binds to a co-stimulatory protein will be 0.1 mg/ml to 10 mg/ml.

Detail Description Paragraph (12):
[0041] Treatment of the graft in a patient or brain dead, beating-heart donor can be accomplished by simply injecting (e.g., intraperitoneally, intravenously, or intra-arterially) or gradually infusing a solution of the co-stimulatory protein-binding molecule and a physiologically acceptable carrier into the donor. For example, the solution can be delivered into a blood vessel of the donor via one of the intravenous lines typically already present in such patients or donors. Generally, the amount of the co-stimulatory protein-binding molecule to be injected will be 1.0 mg to 500 mg, preferably, 5 mg to 50 mg at a concentration of 0.1 .mu.g/ml to 5 mg/ml. When treating grafts in brain dead, beating-heart donors, 0.1 to 1.0 hour of incubation prior to removal of the graft is generally sufficient for inhibiting rejection of the graft in a patient.

Detail Description Paragraph (13):
[0042] Inhibition of Graft Rejection by Treating the Graft in the Donor.

Detail Description Paragraph (14):
[0043] More generally, the invention features any treatment of a graft prior to its removal from a brain-dead, beating-heart donor to inhibit subsequent rejection of the graft in a patient (i.e., recipient). For example, rejection of the graft can be inhibited by modifying, eliminating, or masking a cell-surface protein of the graft. The cell-surface protein can be an antigen which, when present on the surface of a cell of the graft, is capable of causing a T-lymphocyte-mediated response in the patient. Similarly, a co-stimulatory protein of the graft can be masked, modified, or eliminated to inhibit the generation of a co-stimulatory signal in T-lymphocytes in the patient. Known masking agents include F(ab').sub.2 fragments of antibodies directed against co-stimulatory proteins (e.g., a B7 protein) or donor cell antigens (e.g., HLA class 1 antigens). Alternatively, rejection can be inhibited by masking an antigen on the surface of the graft with the use of a soluble host T-cell receptor(s) (i.e., the patient's T-cell receptor) which binds an antigenic site(s) on the graft that would otherwise interact with the patient's T-cells in vivo. Also useful are synthetic organic molecules which mimic the antigen-binding properties of T-cell receptors. If desired, the cell bearing a co-stimulatory protein can be lysed with a chimeric molecule which has (i) a polypeptide which binds to a co-stimulatory protein of antigen-presenting cells fused to (ii) a polypeptide which has a lytic Fc region of an IgG molecule and which lacks a variable region of an IgG heavy chain.

Detail Description Paragraph (15):
[0044] A detailed discussion of methods for masking, eliminating, or modifying a cell-surface antigen is provided in U.S. Pat. No. 5,283,058, hereby incorporated by reference. The methods described therein are also appropriate for masking, eliminating, or modifying a cell-surface co-stimulatory protein. In this aspect of the invention, the graft is treated in the brain-dead, beating-heart donor by perfusion of a solution of the desired masking, eliminating, or modifying agent and a physiologically acceptable carrier into the graft. The graft can also be treated by injecting into the donor (e.g., intraperitoneally, intravenously, or intra-arterially) a solution of a molecule which binds to or co-stimulatory protein or an antigen of antigen-presenting cells.

Detail Description Paragraph (17):
[0046] Animals: Six to eight week old male B6AF1, DBA/2J, and C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, Me.) and housed under standard conditions both before and after transplantation.

Detail Description Paragraph (36):
[0065] Islet Cell Allograft Treatment with (NL) CTLA-4/Fc: To demonstrate that (NL) mCTLA-4/Fc could be incubated with grafts in vitro prior to transplantation to block B7-mediated rejection by donor tissues, crude islet cell isolates were harvested from DBA/2J mice by collagenase digestion and ficoll density gradient separation, as was previously described (Gloth et al., 1986, Transplantation 42:387). Approximately 300-400 islets per transplant were incubated at 37.degree. C. for 1 hour with either media alone, control protein (mIgG3 at 10 .mu.g/ml in RPMI), or (NL) mCTLA-4/Fc (at 10 .mu.g/ml in RPMI). The cells were then pelleted and injected under the left renal capsule of B6AF1 recipients that had been rendered diabetic 7 days earlier by a single intraperitoneal injection of streptozotocin (225 mg/kg). The islet cell recipients were not systemically immunosuppressed. Graft function was monitored by tail blood glucose measurements using the Chemstrip bG and Accu-Chek III blood glucose monitor system (Boehringer Mannheim, Indianapolis, Ind.); other art-recognized methods of measuring blood glucose levels can also be used. Post-transplant primary graft function was defined by a blood glucose level of less than 11.1 mmol/L, and subsequent graft failure was defined by consistent blood glucose levels that were greater than 16.5 mmol/L. To detect graft tolerance, animals with functioning grafts were challenged after 120 days after transplantation with an intraperitoneal injection of 5.times.10.sup.7 irradiated (3000 Rad) donor splenocytes (Shizuru et al., 1987, Science 237:278).

Detail Description Paragraph (37):

[0066] All islet grafts (n=24) that were treated with (NL) mCTLA-4/Fc displayed signs of primary graft function by the sixth day after transplantation. Of these 24 grafts, 10 (42%) went on to exhibit signs of long term (i.e., >150 days) engraftment (FIG. 7) in untreated allogeneic recipient hosts. In order to determine whether graft tolerance was achieved through treating the islet grafts with CTLA-4/Fc, hosts bearing long-term functioning islet grafts (i.e., >150 days) were challenged with donor spleen cells. Of these animals, 50% (3 out of 6) tolerated their grafts. In control experiments, islets were treated with mIgG3. Murine IgG3 proteins do not engage murine Fc.gamma.RI, and they weakly activate complement as compared with mIgG2a isotypes (Paul, 1993, In: Fundamental Immunology, Raven Press). Moreover, IgG immunoglobulins only effect CDC activity as multimeric complexes, while monomeric IgG can bind Fc receptors (Burton, 1985, Molecular Immunology, 7:445). Therefore, a monoclonal mIgG3, which does not bind B7, was chosen as a control ligand for the (NL) mCTLA-4/Fc chimeric protein. All of the IgG3-treated islet grafts (n=9) demonstrated primary graft function, and 89% were acutely rejected (FIG. 7). Islets which were treated with medium alone (n=10) showed signs of primary graft function and were acutely rejected by day 44 (FIG. 7). Thus, only the CTLA-4/Fc-treated grafts were tolerated, and the incubation period of 1 hour for islet graft treatment in the presence of (NL) CTLA-4/Fc was sufficient to lead to significant engraftment.

Detail Description Paragraph (38):
[0067] Immunohistochemistry: The left kidney containing the islet cell graft of a tolerant animal (i.e., an animal in which the graft was functioning at 200 days after transplantation and at 50 days after donor spleen cell challenge) was removed and embedded in OCT compounds. Serial frozen sections were either fixed in cold acetone for immunocytochemistry or fixed in methanol for hematoxylin and eosin staining. Immunohistology was performed with conventional methods (see, e.g., Boegen et al., 1993, J. Immunol. 150(10):4197). Briefly, 0.3 .mu.m sections were sequentially blocked with mouse serum, avidin, and biotin, then quenched with H.sub.2O.sub.2, and then incubated with rat anti-mouse CD4 or CD8 mAbs for 45 minutes in 0.05 M Tris buffer (pH 7.6) at room temperature. Binding of antibodies was detected with a biotinylated rabbit anti-rat mAb and avidin-HRPO complex, using diaminobenzidine for detection of enzyme activity. Negative controls were processed as above with the exclusion of the primary antibody. Sections were counter-stained with methyl green ($C_{27}H_{35}BrClN_3ZnCl_2$).

Detail Description Paragraph (39):
[0068] Histologic analysis of islet cell allografts harvested from tolerant animals (i.e., >day 150 post transplantation and >day 50 post donor spleen cell challenge) demonstrated a dense molecular cell infiltrate surrounding, but not invading, the islets (FIGS. 8A-D). The majority of these cells were $CD4^+$ cells; a significant number (approximately 30% of the level of $CD4^+$ cells) of $CD8^+$ cells were also detected. These data indicate that, while treatment of islet grafts with (NL) mCTLA-4/Fc does not eliminate cellular responses to the graft, the responding mononuclear cells do not aggressively infiltrate the islet tissue. Aggressive infiltration leads to islet cell destruction, and such infiltration is characteristic of rejection (see, e.g., O'Connell et al., 1993, J. Immunol. 150:1093).

CLAIMS:

1. A method for inhibiting rejection of a graft containing a cell which expresses a co-stimulatory protein in a patient, said method comprising treating said graft in said patient with a molecule which binds to a co-stimulatory protein of antigen-presenting cells to inhibit activation of host T-cells by said graft, wherein said molecule is a molecule other than lytic CTLA-4/Fc.

3. The method of claim 1, wherein said graft is further treated ex vivo.

4. The method of claim 1, wherein said graft is further treated in a brain-dead, beating-heart donor.

16. The method of claim 1, wherein said graft is treated with a molecule comprising CD2 and with a molecule comprising CTLA-4.

18. A method for inhibiting rejection of a graft containing a cell which expresses a

co-stimulatory protein in a patient, said method comprising treating said graft outside of said patient with a molecule which binds to a co-stimulatory protein of antigen-presenting cells to inhibit activation of host T-cells by said graft.

22. The method of claim 18, wherein said graft is treated ex vivo.

23. The method of claim 18, wherein said graft is treated in a brain-dead, beating-heart donor.

24. The method of claim 18, wherein said graft is further treated in a patient.

36. The method of claim 18, wherein said graft is treated with a molecule comprising CD2 and with a molecule comprising CTLA-4.

37. The method of claim 36, wherein said treatment with said molecule comprising CTLA-4 occurs simultaneously with said treatment of said graft with said chimeric molecule comprising CD2.

39. A method for inhibiting the rejection of a graft in a patient, comprising treating the brain-dead, beating-heart donor of said graft prior to removal of said graft from said donor to render said graft less susceptible to rejection by said patient.

40. The method of claim 39, wherein said treatment of said graft comprises modifying, eliminating, or masking an antigen of said graft which, when present on the surface of a cell of said graft, is capable of causing a T-lymphocyte-mediated response in said patient.

41. The method of claim 40, wherein said masking comprises treating said graft with a non-lytic masking agent which comprises an antibody F(ab').sub.2 fragment which is capable of forming a complex with said antigen on said cell.

42. The method of claim 39, wherein said treatment of said graft comprises modifying, eliminating, or masking a co-stimulatory protein which when present on the surface of a cell of said graft is capable of causing a co-stimulatory signal in said patient.

43. The method of claim 42, wherein said masking comprises treating said graft with a non-lytic masking agent which comprises an antibody F(ab').sub.2 fragment which is capable of forming a complex with said co-stimulatory protein on said cell.

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DOCUMENT-IDENTIFIER: US 20020142000 A1
TITLE: Anti-CD3 immunotoxins and therapeutic uses therefor

Abstract Paragraph (1):
Recombinant immunotoxin polypeptides are described comprising a CD3-binding domain and a Pseudomonas exotoxin mutant, and in particular, comprising a single chain (sc) Fv as the CD3-binding moiety. A preferred species of the invention comprises scFv(UCHT-1)-PE38. Also disclosed are methods for the preparation of said immunotoxins; functionally equivalent immunotoxins which are intermediates in the preparation of the immunotoxins of the invention, as well as polynucleotide and oligonucleotide intermediates; methods for the prevention and/or treatment of transplant rejection and induction of tolerance, as well as treatment of autoimmune and other immune disorders, using the immunotoxins or pharmaceutically acceptable salts thereof; and pharmaceutical compositions comprising the immunotoxins or pharmaceutically acceptable salts thereof.

Summary of Invention Paragraph (7):
[0005] The adaptive immune mechanisms described above constitute a major impediment to successful organ transplantation. When tissues containing nucleated cells are transplanted from a donor to a graft recipient, T-cell responses in the recipient to the typically highly polymorphic MHC molecules of the graft almost always trigger an immediate T-cell mediated response against the grafted organ. The use of potent immunosuppressives such as cyclosporin A and FK-506 to inhibit T cell activation has increased graft survival rates dramatically, but with certain disadvantages, including life-long dependence on the drug by the graft recipient.

Summary of Invention Paragraph (8):
[0006] Development of improved means of immunosuppression in patients receiving organ transplants, or suffering from T-cell mediated immune disease, has been a constant objective in the field of transplantation. A particular objective of workers in the art is development of a therapeutic agent capable of inducing donor-specific immunologic tolerance in a patient, and thereby freeing the patient from otherwise continuous dependence on immunosuppressives.

Summary of Invention Paragraph (9):
[0007] The term "immunological tolerance" refers to a state of unresponsiveness by the immune system of a patient subject to challenge with the antigen to which tolerance has been induced. In the transplant setting, in particular, it refers to the inhibition of the graft recipient's ability to mount an immune response which would otherwise occur in response to the introduction of non-self MHC antigen of the graft into the recipient. Induction of immunological tolerance can involve humoral, cellular, or both humoral and cellular mechanisms.

Summary of Invention Paragraph (10):
[0008] Systemic donor-specific immunological tolerance has been demonstrated in animal models as well as in humans through chimerism as a result of conditioning of the patient through total body irradiation or total lymphoid irradiation, prior to bone marrow transplantation with donor cells, Nikolic, B. and Sykes, M. (1997) Immunol. Res. 16: 217-228.

Summary of Invention Paragraph (11):
[0009] However, there remains a critical need for a conditioning regimen for allogeneic bone marrow transplantation that will result in stable mixed multilineage

allogeneic chimerism and long-term donor-specific tolerance, in the absence of radiation. Hematologic abnormalities including thalassemia and sickle cell disease, autoimmune states, and several types of enzyme deficiency states, have previously been excluded from bone marrow transplantation strategies because of morbidity associated with conditioning to achieve fully allogeneic bone marrow reconstitution. Conditioning approaches which do not involve radiation may significantly expand the application of bone marrow transplantation for non-malignant diseases.

Summary of Invention Paragraph (12):
[0010] Immunotoxins comprising an antibody linked to a toxin have been proposed for the prophylaxis and/or treatment of organ transplant rejection and induction of immunological tolerance. For example, a chemically conjugated diphtheria immunotoxin directed against rhesus CD3.epsilon., i.e. FN18-DT390, has been used in primate models of allograft tolerance and also in primate islet concordant xenograft models, see Knechtle et al.(1997) Transplantation 63:1, Neville et al. (1996) J. Immunother. 19: 85; Thomas et al. (1997) Transplantation 64: 124; Contreras et al. (1998) Transplantation 65: 1159-1169. Additionally, a chemically coupled Pseudomonas immunotoxin, LMB-1 B3(Lys)-PE38, has been used in clinical trials against advanced solid tumors, Pai, L. H. and I. Pastan, Curr. Top. Microbiol. Immunol. 234:83-96 (1998). However, product heterogeneity is a significant practical difficulty associated with chemically conjugated immunotoxins.

Summary of Invention Paragraph (13):
[0011] A single chain recombinant immunotoxin comprising the variable region of an anti-CD3 antibody, UCHT-1 and a diphtheria toxin, has been proposed as a therapeutic agent, see WO 96/32137, WO 98/39363. However, early vaccination of the general population against diphtheria raises concerns about pre-existing antibodies to the toxin in many patients. Alternately, a recombinant immunotoxin comprising anti-Tac linked to PE38 is also proposed as a prophylaxis and treatment against organ transplantation and autoimmune disease, see Mavroudis et al. (1996). Bone Marrow Transplant. 17: 793.

Summary of Invention Paragraph (14):
[0012] It has been an object to achieve a recombinant immunotoxin having directed toxic effect at high levels against T cells, which thereby provides improvements in the prophylaxis or treatment of transplant rejection and in the induction of immunologic tolerance, as well as in the treatment or prevention of graft versus host disease (GVHD), autoimmune disease, and other T-cell mediated diseases or conditions.

Summary of Invention Paragraph (16):
[0014] We have now discovered that recombinant fusions of a CD3-binding domain and a Pseudomonas exotoxin A mutant provide an immunotoxin having potent anti-T cell effect. The immunotoxins of the invention provide improvements in the clinical treatment or prevention of transplant rejection, graft-versus-host disease (GVHD), T-cell mediated autoimmune disease, T-cell leukemias, or lymphomas which carry the CD3 epitope, acquired immune deficiency syndrome (AIDS), and other T-cell mediated diseases and conditions.

Summary of Invention Paragraph (18):
[0015] The present invention is directed to isolated recombinant immunotoxins comprising a CD3-binding domain and a Pseudomonas exotoxin A component, and pharmaceutically acceptable salts thereof; to in vivo and ex vivo methods for the treatment and prophylaxis of organ transplantation rejection and graft-versus-host disease, and for the induction of immunologic tolerance, as well as for treatment or prophylaxis of auto-immune diseases, AIDS and other T-cell mediated immunological disorders, and T-cell leukemias or lymphomas, using the immunotoxins or pharmaceutically acceptable salts thereof; and to pharmaceutical compositions comprising the novel immunotoxins or their pharmaceutically acceptable salts.

Detail Description Paragraph (49):
[0080] (3) BC-3 (Fred Hutchinson Cancer Research Institute) (used in Phase I/II trials of GvHD) (Anasetti, et al., (1992) Transplantation 54: 844).

Detail Description Paragraph (161):
[0192] It is within the scope of the present invention to provide a prophylaxis or

treatment of T-cell mediated diseases or conditions by administering immunotoxin to a patient in vivo for the purpose of systemically killing T cells in the patient, and as a component of a preparation or conditioning regimen or induction tolerance treatment in connection with bone marrow or stem cell transplantation, or solid organ transplantation from either a human (allo-) or non-human (xeno-) source.

Detail Description Paragraph (165):
[0196] For example, the immunotoxins can usefully be administered to a patient who is or will be a recipient of an allotransplant (or xenotransplant), in order to effect T-cell depletion in the patient and thereby prevent or reduce T-cell mediated acute or chronic transplant rejection of the transplanted allogeneic (or xenogeneic) cells, tissue or organ in the patient, or to permit the development of immunological tolerance to the cells, tissue or organ.

Detail Description Paragraph (166):
[0197] Preferably, when administered in vivo to prevent or treat organ transplant rejection, it is desirable that the immunotoxin be administered to the patient over time in several doses. In general, it is preferred that at least the first dose precede the transplant surgery (preferably as long in advance as possible), and a subsequent dose or doses begin at the time of or shortly following the surgery.

Detail Description Paragraph (167):
[0198] The immunotoxins can be administered in vivo either alone or in combination with other pharmaceutical agents effective in treating acute or chronic transplant rejection including cyclosporin A, cyclosporin G, rapamycin, 40-O-2-hydroxyethyl-substituted rapamycin (RAD), FK-506, mycophenolic acid, mycophenolate mofetil (MMF), cyclophosphamide, azathioprene, brequinar, leflunamide, mizoribine, deoxyspergualines, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY 720), corticosteroids (e.g., methotrexate, prednisolone, methylprednisolone, dexamethasone), or other immunomodulatory compounds (e.g., CTLA4-Ig); anti-LFA-1 or anti-ICAM antibodies, or other antibodies that prevent co-stimulation of T cells, for example antibodies to leukocyte receptors or their ligands (e.g., antibodies to MHC, CD2, CD3, CD4, CD7, CD25, CD28, B7, CD40, CD45, CD58, CD152 (CTLA-4), or CD 154 (CD40 ligand).

Detail Description Paragraph (168):
[0199] In particular, prolonged graft acceptance and even apparent immunologic tolerance can be achieved by combined administration of an anti-CD3 immunotoxin of the invention and a spergualin derivative, such as a deoxyspergualine compound, or other spergualin analog, and this invention in a preferred embodiment comprises the combined administration of anti-CD3 immunotoxin and a deoxyspergualine compound in a tolerance induction regimen, see for example, Eckhoff et al., abstract presented to American Society of Transplant Surgeons, May 15, 1997, and Contreras, et al., (1998) Peritransplant tolerance induction with anti-CD3 immunotoxin : A matter of proinflammatory cytokine control. Transplantation 65: 1159, both incorporated by reference. The term "deoxyspergualine compound" includes 15-deoxyspergualin (referred to as "DSG", and also known as gusperimus), i.e. i.e. N-[4-(3-aminopropyl) aminobutyl]-2-(7-N-guanidinoh- eptanamido)-2-hydroxyethanamide, and its pharmaceutically acceptable salts, as disclosed in U.S. Pat. No. 4,518,532, incorporated by reference; and in particular (-)-15-deoxyspergualin and its pharmaceutically acceptable salts as disclosed in U.S. Pat. No. 4,525,299, incorporated by reference. The optically active (S)-(-) or (R)-(+)-15-deoxyspergualin isomers and salts thereof are disclosed in U.S. Pat. No. 5,869,734 and EP 765,866, both incorporated by reference; and the trihydrochloride form of DSG is disclosed in U.S. Pat. No. 5,162,581, incorporated by reference.

Detail Description Paragraph (178):
[0209] By "combined administration" is meant treatment of the organ transplant recipient with both an anti-CD3 immunotoxin of the invention and the spergualin derivative or analog.

Detail Description Paragraph (180):
[0211] The total dose of the anti-CD3 immunotoxin is preferably given over 2-3 injections, the first dose preceding the transplant by the maximal time practicable, with subsequent injections spaced by intervals of, for example, about 24 hours.

Detail Description Paragraph (181):
[0212] The immunotoxin is preferably administered prior to transplant and at the time of and/or following transplant.

Detail Description Paragraph (182):
[0213] In allotransplantation, administration of the anti-CD3 immunotoxin preferably precedes transplant surgery by about 2-6 hours, whereas for xenotransplantation or living related allotransplantation, the first anti-CD3 immunotoxin injection may precede transplantation by as much as one week, see for example, Knechtle, S. J., et al. (1997) FN18-CRM9 immunotoxin promotes tolerance in primate renal allografts. Transplantation 63: 1.

Detail Description Paragraph (183):
[0214] In a tolerance induction regimen, the immunotoxin treatment is preferably curtailed no later than about 14 days, and preferably on about day 7 , or on day 5, or even on day 3, post-transplant.

Detail Description Paragraph (184):
[0215] The spergualin derivative or analog may be administered prior to transplant, at the time of transplant, and/or following transplant. The length of treatment either before or after transplant may vary.

Detail Description Paragraph (185):
[0216] In a tolerance induction regimen, the treatment with spergualin derivative or analog compound is preferably withdrawn not later than about 120 days following transplant, and more preferably after about 60 days post-transplant, and more preferably after about 30 days, and even more preferably not later than 14, or even about 10 days, post-transplant.

Detail Description Paragraph (186):
[0217] Thus, the term "combined administration" includes within its scope a treatment regimen wherein, for example, one or more doses of immunotoxin is/are administered prior to the transplant, followed by one or more doses commencing at around the time of transplant; together with administration of the spergualin derivative or analog also prior to and/or at the time of transplant, and typically continuing after transplant.

Detail Description Paragraph (187):
[0218] Corticosteroids such as methylprednisolone may be incorporated into the combined administration regimen. For example, steroid administration may commence prior to transplant, and may continue with one or more doses thereafter.

Detail Description Paragraph (191):
[0222] Additional steroids may be administered at the time of the anti-CD3 immunotoxin injections, for example as a decreasing regimen of methylprednisone, such as 7 mg/kg on the day of the transplant surgery, 3.5 mg/kg at +24 hours, and 0.35 mg/kg at +48 hours. Alternatively, the steroid dosage may be held constant, for example treatment with 40 mg/kg of prednisone at the time of immunotoxin injection. It is understood that the exact amount and choice of steroid can vary, consistent with standard clinical practice.

Detail Description Paragraph (196):
[0227] In the practice of the above combination therapy and the other methods of this invention in the context of xenotransplantation, and especially where the transplant recipient is human, the donor cells, tissues or organs are preferably porcine, and are most preferably recruited from transgenic, e.g., human DAF expressing, pigs.

Detail Description Paragraph (197):
[0228] In another embodiment of the methods of the invention, the immunotoxins can be administered in vivo to a bone marrow recipient for prophylaxis or treatment of host-versus-graft disease through killing of host (i.e. bone marrow transplant recipient) T cells. Marrow transplants become necessary in the treatment of certain diseases, such as leukemia, aplastic anemia or certain genetic disorders, in which the patient's own marrow is severely flawed or where total body irradiation or

chemotherapy have destroyed the patient's hematopoietic system. Absent reconstitution of the hematopoietic system by bone marrow transplantation, the patient becomes severely immunodepressed and susceptible to infection.

Detail Description Paragraph (198):
[0229] Stable engraftment of donor allogeneic bone marrow depends in large part on MHC matching between donor and recipient. In general, mismatching only to the extent of one or two antigens is tolerable in bone marrow transplantation because of rejection of the disparate bone marrow graft by recipient T cells. (Also, graft versus host disease, discussed below, is very severe when there are greater disparities.) In addition, even minor mismatching conventionally necessitates conditioning of the recipient by lethal or sublethal doses of total body irradiation or total lymphoid irradiation to deplete recipient T-cells. This requirement for irradiation of the bone marrow transplant patient which renders the patient totally or nearly immunoincompetent poses a significant limitation on clinical application of bone marrow transplantation to a variety of disease conditions in which it is potentially useful, including solid organ or cellular transplantation, sickle cell anemia, thalassemia and aplastic anemia.

Detail Description Paragraph (200):
[0231] Thus this invention provides in another of its aspects, a method for conditioning a bone marrow transplant patient prior to engraftment in the patient of donor bone marrow and/or stem-cell enriched peripheral blood cells, comprising administration of a T-cell depleting effective amount of immunotoxin to the patient. The immunotoxin effects reductions in the T cell population in the patient and thereby exerts a prophylaxis against host (i.e. the patient's) rejection of the donor bone marrow graft. Methods of obtaining donor compositions enriched for hematopoietic stem cells are disclosed in U.S. Pat. No. 5,814,440, No. 5,681,559, No. 5,677,136, and No. 5,061,620, all incorporated by reference.

Detail Description Paragraph (201):
[0232] Graft-versus-host disease (GVHD), in particular, is a sometimes fatal, often debilitating complication of allogeneic bone marrow transplant which is mediated primarily, if not exclusively, by T lymphocytes. GVHD is caused by donor T cells which are acquired in the graft by the bone marrow recipient and which develop an immune response against the host. GVHD typically results from incomplete immunologic matching of donor and recipient Human leukocyte antigens (HLA).

Detail Description Paragraph (202):
[0233] Accordingly, this invention also contemplates a method of prophylaxis or treatment of GVHD in a bone marrow transplant patient, comprising administration of an immunotoxin of the invention to the patient during the early post-transplant period, or when symptoms of GVHD become manifest, in an amount sufficient to effect reductions in levels of T cells in the host (i.e. patient), including both donor and host T cells. The early depletion of donor and host T-cells also facilitates the development of allogeneic chimerism; that is, the T cells which are given space to mature following host T-cell ablation by immunotoxin are rendered tolerant of both donor and host antigens and do not participate in graft versus host rejection. By "early post-transplant period" is meant a period of one or more days up to about two weeks following bone marrow transplantation.

Detail Description Paragraph (206):
[0237] Additionally, the anti-CD3 immunotoxin can be administered to patients to treat conditions or diseases in instances in which chronic immunosuppression is not acceptable, e.g., by facilitating islet or hepatocyte transplants in patients with diabetes or metabolic diseases, respectively. Diseases and susceptibilities correctable with hepatocyte transplants include hemophilia, .alpha.1-antitrypsin insufficiencies, and hyperbilirubinemias.

Detail Description Paragraph (207):
[0238] In the above methods of the invention, the patient is preferably human and the donor may be allogeneic (i.e. human) or xenogeneic (e.g., swine). The transplant may be an unmodified or modified organ, tissue or cell transplant, e.g., heart, lung, combined heart-lung, trachea, liver, kidney, pancreas, Islet cell, bowel (e.g., small bowel), skin, muscles or limb, bone marrow, oesophagus, cornea or nervous tissue

transplant.

Detail Description Paragraph (210):
[0241] Preferably, in the treatment or prophylaxis of GVHD accompanying bone marrow transplantation, the immunotoxin is administered to the bone marrow transplant recipient in an amount sufficient to reduce the total T-cell population (i.e. donor plus recipient T cells) present in the patient blood and lymph nodes immediately following bone marrow transplantation by at least about 50% and more preferably at least about 80%, and even more preferably at least about 95% (e.g., 99%), i.e. by at least 2 logs (e.g., by 2-3 logs).

Detail Description Paragraph (211):
[0242] A suitable dosing regimen for a bone marrow recipient, to treat or prevent host versus graft disease and/or GVHD, may comprise administration of immunotoxin immediately prior to, and/or immediately following bone marrow transplantation on each alternating day over the course of six days after transplant, to bring the total dose to about 10-500 .mu.g/kg, and more preferably 200-300 .mu.g/kg.

Detail Description Paragraph (215):
[0246] Depletion of T-cell numbers by 2 logs, by a chemically conjugated immunotoxin comprised of an anti-rhesus CD3 monoclonal antibody conjugated to a cell binding domain-deleted form of diphtheria toxin, has been shown to be associated with transplantation tolerance to renal allografts in rhesus monkeys (Thomas et al., 1997, Transplantation 64:124-135; Knechtle et al., 1997, Transplantation 63:1-6).

Detail Description Paragraph (220):
[0251] For example, the polypeptide, scFv(UCHT-1)-PE38 of Example 1, may be administered to a kidney transplant patient starting just prior to transplantation and continuing, post-transplant, over the course of a week in daily or alternate day dosing, at a dose of about 0.3-10 mg per week of polypeptide in the average patient (70 kg). After the first week post-transplant, the treatment regimen may be reduced to alternating weeks, with dosages ranging from 0.1 mg to 1 mg of polypeptide per week in the average patient. It is expected, however, that immunotoxin treatment shall be curtailed at five weeks after transplant, and more typically at three weeks, or even at one week post-transplant.

Detail Description Paragraph (223):
[0254] In one aspect, the immunotoxins can be used in a method for prophylaxis of organ transplant rejection, wherein the method comprises perfusing the donor organ (e.g., heart, lung, kidney, liver) prior to transplant into the recipient with a composition comprising a T-cell depleting effective amount of immunotoxin, in order to purge the organ of sequestered donor T-cells.

Detail Description Paragraph (233):
[0264] According to still another embodiment of the invention, the immunotoxins can be utilized ex vivo for purposes of effecting T cell depletion from a donor cell population as a prophylaxis against graft versus host disease, and induction of tolerance, in a patient to undergo a bone marrow transplant. Such a method comprises the steps of:

Detail Description Paragraph (237):
[0268] By virtue of T-cell depletion from the donor inoculum, the donor T cells which mature following engraftment are rendered immunologically tolerant of the host and will not initiate graft versus host rejection.

Detail Description Paragraph (241):
[0272] In a further aspect, the above ex vivo therapeutic methods can be combined with in vivo administration of immunotoxin, to provide improved methods of treating or preventing rejection in bone marrow transplant patients, and for achieving immunological tolerance.

Detail Description Paragraph (242):
[0273] For example, a method comprising both in vivo and ex vivo administration of an immunotoxin of the invention for the prophylaxis and/or treatment of host versus graft disease and/or graft versus host disease in a patient to undergo a bone marrow

transplant comprises the steps of:

Detail Description Paragraph (247):
[0278] The in vivo and ex vivo methods of the invention as described above are suitable for the treatment of diseases curable or treatable by bone marrow transplantation, including leukemias, such as acute lymphoblastic leukemia (ALL), acute nonlymphoblastic leukemia (ANLL), acute myelocytic leukemia (AML), and chronic myelocytic leukemia (CML), cutaneous T-cell lymphoma, severe combined immunodeficiency syndromes (SCID), osteoporosis, aplastic anemia, Gaucher's disease, thalassemia, mycosis fungoides (MF), Sezany syndrome (SS), and other congenital or genetically-determined hematopoietic abnormalities.

Detail Description Paragraph (248):
[0279] In particular, it is also within the scope of this invention to utilize the immunotoxins as agents to induce donor-specific and antigen-specific tolerance in connection with allogeneic or xenogeneic cell therapy or tissue or organ transplantation. Thus, the immunotoxin can be administered as part of a conditioning regimen to induce immunological tolerance in the patient to the donor cells, tissue or organ, e.g., heart, lung, combined heart-lung, trachea, liver, kidney, pancreas, Islet cell, bowel (e.g., small bowel), skin, muscles or limb, bone marrow, oesophagus, cornea or nervous tissue.

Detail Description Paragraph (249):
[0280] Systemic donor-specific transplantation tolerance has been transiently achieved in MHC-mismatched animal models as well as in humans through chimerism as a result of total lymphoid irradiation of a recipient followed by bone marrow transplantation with donor cells. The reconstituted animals exhibit stable mixed multilineage chimerism in their peripheral blood, containing both donor and recipient cells of all lymphohematopoietic lineages, including T cells, B cells, natural killer cells, macrophages, erythrocytes and platelets. Furthermore, the mixed allogeneic chimeras display donor-specific tolerance to donor-type skin grafts, while they readily reject third-party grafts. Donor-specific tolerance is also confirmed by in vitro assays in which lymphocytes obtained from the chimeras are shown to have diminished proliferative and cytotoxic activities against allogeneic donor cells, but retain normal immune reactivity against third-party cells.

Detail Description Paragraph (250):
[0281] Thus the present invention further contemplates a method of conditioning a patient to be transplanted with donor cells, or a tissue or organ. The method comprises the steps of:

Detail Description Paragraph (254):
[0285] (d) transplanting the donor cells, tissue or organ into the patient.

CLAIMS:

22. A method for treatment or prophylaxis of organ transplantation rejection in a transplant patient comprising administering to the patient a therapeutically effective amount of a recombinant immunotoxin polypeptide or its pharmaceutically acceptable salt according to claim 1.

25. A method for treatment or prophylaxis against graft versus host disease in patient to undergo a bone marrow transplant comprising: (a) providing an inoculum comprising isolated bone marrow and/or stem cell-enriched peripheral blood cells of a suitable donor treated with a T-cell depleting effective amount of a recombinant immunotoxin polypeptide or its pharmaceutically acceptable salt according to claim 1; and (b) transplanting the inoculum into the patient.

26. A method for the treatment or prophylaxis or treatment of transplant rejection in a patient to undergo a bone marrow transplant comprising: (a) reducing the levels of viable CD3-bearing cell population in the patient; (b) providing an inoculum comprising isolated bone marrow and/or stem cell-enriched peripheral blood cells of a suitable donor treated with a T-cell depleting effective amount of a recombinant immunotoxin polypeptide or its pharmaceutically acceptable salt according to claim 1; and (c) introducing the inoculum into the patient, and thereafter optionally

administering a recombinant immunotoxin polypeptide according to claim 1 to the patient to further deplete donor and patient T cells.

27. A method of conditioning a patient to be transplanted with cells, or a tissue or organ of a donor, the method comprising: (a) depleting the CD3-bearing cell population in the patient; (b) providing an inoculum comprising isolated bone marrow and/or stem-cell enriched peripheral blood cells of the donor treated with a T-cell depleting effective amount of a recombinant immunotoxin polypeptide or its pharmaceutically acceptable salt according to claim 1; (c) introducing the inoculum into the patient; and (d) transplanting the donor cells, tissue or organ into the patient.

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DOCUMENT-IDENTIFIER: US 20020091142 A1
TITLE: Alpha4beta1 and alpha4beta7 integrin inhibitors

Abstract Paragraph (2):
as defined and their pharmaceutically acceptable salts are inhibitors of .alpha..sub.4.beta..sub.1 and/or .alpha..sub.4.beta..sub.7 integrins, and are useful in inhibiting cell adhesion and in the therapeutic or prophylactic treatment of transplant rejection and inflammatory and autoimmune diseases.

Summary of Invention Paragraph (1):
[0002] The present invention relates to compounds which inhibit .alpha..sub.4-mediated cellular adhesion, and as such are useful in the treatment of various conditions such as acute or chronic organ transplant rejection, inflammatory bowel disease, asthma, diabetes, and rheumatoid arthritis.

Summary of Invention Paragraph (67):
[0068] The ability of the compounds of formula I to inhibit .alpha..sub.4.beta..sub.7 and/or .alpha..sub.4.beta..sub.7 associated cell adhesions makes them useful for treating, ameliorating, or preventing a variety of inflammatory, immune and autoimmune diseases. Preferably the diseases to be treated with the methods of this invention are selected from respiratory disorders (such as asthma), arthritis, psoriasis, transplantation rejection, multiple sclerosis, type I diabetes, and inflammatory bowel disease, stem cell mobilization and engraftment, and sickle cell anemia.

Summary of Invention Paragraph (68):
[0069] The compounds of formula I are also useful in transplantation surgery; specifically, for the treatment of xenograft and allograft rejection, both chronic and acute, for the induction of tolerance to donor cells, tissues or organs, or in treating ischemia reperfusion/injury.

Summary of Invention Paragraph (69):
[0070] The compound of formula I may be administered optionally prior to the transplant operation, and at the time of and/or following the transplant operation.

Summary of Invention Paragraph (70):
[0071] When used for the suppression of organ transplant rejection, a compound of formula I may be administered as the sole active ingredient or together with other drugs in immunomodulating regimens or other anti-inflammatory agents effective in treating acute or chronic rejection. For example, the compounds of formula I may be used in combination with cyclosporins, rapamycins or ascomycins, or their immunosuppressive analogs, e.g. cyclosporin A, cyclosporin G, FK-506, rapamycin, 40-O-(2-hydroxy)ethyl-rapamycin (RAD), etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; brequinar; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil (MMF); deoxyspergualins (e.g., 15-deoxyspergualine) and analogs, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride, corticosteroids (e.g., methotrexate, prednisolone, methylprednisolone, dexamethasone), or other immunomodulatory compounds (e.g., CTLA4-lg); anti-LFA-1 or anti-lCAM antibodies, or antibodies to leukocyte receptors or their ligands (e.g., antibodies to MHC, CD2, CD3, CD4, CD7, CD25, CD28, B7, CD40, CD45, CD58, CD152 (CTLA-4), or CD 154 (CD40 ligand).

Summary of Invention Paragraph (78):
[0079] (A) the use of a compound of the invention, i.e. a compound of formula I or a pharmaceutically acceptable salt thereof, as hereinbefore described, for the

preparation of a medicament for the treatment of inflammatory, immune or autoimmune diseases, particularly arthritis, transplant rejection or inflammatory airways diseases, especially asthma; and

Summary of Invention Paragraph (79):
[0080] (B) a method of treating an inflammatory, immune or autoimmune disease, particularly arthritis, transplant rejection or an inflammatory airways disease, especially asthma, which comprises administering to a mammal, particularly a human, in need of such treatment a compound of the invention as hereinbefore described.

Summary of Invention Paragraph (85):
[0086] The pharmaceutical compositions of the present invention comprise a compound of formula I, or a pharmaceutically acceptable salt thereof, as an active ingredient, and may also contain a pharmaceutically acceptable carrier and, optionally, other therapeutically active ingredients. The invention includes such compositions for use in the treatment of an inflammatory, immune or autoimmune disease, particularly arthritis, transplant rejection or an inflammatory airways disease, especially asthma.

Summary of Invention Paragraph (91):
[0092] The compositions containing a compound of this invention may also comprise an additional agent selected from the group consisting of cortiocosteroids, bronchodilators, antiasthmatics (mast cell stabilizers), anti-inflammatories, antirheumatics, immunosuppressants, anti-metabolites, immunonodulators, antipsoriatics, and antidiabetics. Specific compounds include theophylline, sulfasalazine and aminosalicylates (anti-inflammatories); or other drugs as mentioned above in connection with suppression of transplant rejection (e.g., cyclosporin, FK-506, and rapamycin (immunosuppressants); cyclophosphamide and methotrexate (antimetabolites) and the like); and interferons (immunomodulators).